Beyond the cloudiness in urinary tract infection: definitions, diagnostics, and strategies for prevention

Bilsen, M.P.

Beyond the cloudiness in urinary tract infection:

Definitions, diagnostics, and strategies for prevention

Manu Bilsen

Beyond the cloudiness in urinary tract infection: definitions, diagnostics, and strategies for prevention

ISBN: 978-94-6506-143-6
Cover design: Guy Vording
Lay-out and design: Ridderprint www.ridderprint.nl
Printing: Ridderprint, www.ridderprint.nl

Financial support for the printing of this thesis has been provided by:
Sysmex Nederland B.V.

Beyond the cloudiness in urinary tract infection:
definitions, diagnostics, and strategies for prevention

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op dinsdag 3 september 2024
klokke 14:30 uur

door

Manu Paul Bilsen
geboren te Amsterdam
in 1993

Promotores

Prof. dr. L.G. Visser

Prof. dr. S.P. Conroy, University College London

Co-promotor

Dr. M.M.C. Lambregts

Leden promotiecommissie

Prof. dr. S.P. Mooijaart

Prof. dr. C.M. Cobbaert

Prof. dr. S.E. Geerlings, Amsterdam University Medical Center

Dr. A. Huttner, University of Geneva

Dr. E.P. van Haarst, Onze Lieve Vrouwe Gasthuis

Voor (en na) Bram

TABLE OF CONTENTS

Chapter 1

General introduction

Epidemiology and burden

Urinary tract infection (UTI) is one of the most commonly encountered bacterial infections worldwide. Current estimates suggest that UTI affects over 400 million people annually, which is likely to be an underestimate of the actual global incidence due to underreporting, particularly in developing countries. [1] In the United States, UTI accounted for 8.6 million ambulatory care visits in 2007, with the majority being primary care office visits (59%) and emergency department visits (23%). [2] Similarly, in the Netherlands, UTI is the most common reason for a primary care consultation, with 149 office visits per 1000 patients (translating to 2.6 million office visits) in 2022 alone. [3]

UTI incidence varies by biological sex and age. Premenopausal women are disproportionately affected, with an incidence as high as 0.7 per person-year in a cohort of sexually-active university students. [4] A second peak occurs after the age of 65 years, when incidence increases with advancing age for both men and women, and is highest in women residing in long-term care facilities (LTCF). [5, 6] Predictive factors for UTI in this population include cognitive and functional impairment, previous UTI and urinary incontinence. [6] Among older women, recurrence rates are high. In a cohort study evaluating one-year recurrence rates in 180 women after an index episode, postmenopausal women had higher recurrence rates compared with premenopausal women (53% versus 36%). [7] Even higher one-year recurrence rates (69% and 79%) were found in a larger randomised trial comparing prophylactic strategies in postmenopausal women. [8]

The high incidence and recurrence rate of UTI place a significant socioeconomic burden on society. Women with recent or recurrent UTI consistently demonstrate reduced quality of life scores across both mental and physical domains, with impairments in activities such as sleep, exercise and sexual intercourse. [9, 10] Beyond direct medical expenses related to doctor's visits, laboratory testing and treatment, there are potential indirect costs if symptoms prevent patients from carrying out work-related tasks. Older adults significantly contribute to excessive healthcare costs, as UTI is the second most common suspected infection requiring hospitalisation in this population. [11] With increasing life expectancy the overall burden of UTI is expected to rise substantially.

Spectrum of disease and definitions

UTI is an umbrella term referring to a wide range of clinical phenotypes that differ in terms of site of infection, duration and severity of symptoms and signs. As highlighted in the previous paragraph, UTI occurs in both men and women of all age groups, and each population is characterised by different risk factors. This variety in clinical phenotypes and patient populations is reflected in the various specialties that encounter patients with UTI, including family medicine, emergency medicine, internal medicine, geriatric medicine, infectious diseases, microbiology, urology, gynaecology, and paediatrics.

Acute cystitis refers to a UTI presumed to be confined to the bladder. This phenotype is predominantly observed in women, possibly due to the shorter distance from the urethra to the perineum. Women typically present with new-onset lower urinary tract symptoms, such as dysuria, frequency, urgency and suprapubic pain, while signs of systemic illness, such as fever and rigors, are absent. Symptoms are self-limiting in the majority of patients (although antimicrobial treatment is often initiated in clinical practice) and progression to upper urinary tract infection is rare. [12, 13]

In men, prostatic involvement is common and may occur through bacterial migration from the urethra, intraprostatic urinary reflux, or from direct inoculation following urogenital instrumentation or transrectal biopsy. [14] In addition to urogenital symptoms, men with acute bacterial prostatitis generally present with systemic signs and symptoms. In a randomised trial evaluating the optimal treatment duration of acute bacterial prostatitis, 17% of the included participants had bacteraemia. [15] Approximately 10% of men with acute bacterial prostatitis develop chronic bacterial prostatitis, which tends to recur despite prolonged antimicrobial treatment. [16]

Acute pyelonephritis indicates an upper urinary tract infection involving the renal pelvis and kidney. Population-based studies show that acute pyelonephritis occurs more frequently in women than in men (annual rate of 15 cases per 10.000 women when combining in- and outpatients) with a notable peak in women aged 15 – 35 years, and a second, gradually increasing incidence after 65 years. [17] Acute pyelonephritis typically manifests with systemic signs and symptoms, flank pain and/or costovertebral angle tenderness, although clinical presentations can vary. [18] Despite high rates of bacteraemia (25-40%) mortality is generally low, with

exceptions for older hospitalised patients, in whom mortality rates can exceed 30%. [19–22]

At the end of the severity spectrum lies urosepsis, defined as a life-threatening organ dysfunction caused by a dysregulated host response to UTI. Surviving Sepsis Campaign data show that urosepsis is the second most common cause of septic shock, second only to a pulmonary source. [23] Despite antimicrobial therapy and supportive care, septic shock has a high mortality rate (32%).

Clearly, UTI is not a single type of infection, and as such it has proven difficult to come up with a single definition. For instance, the Centers for Disease Control and Prevention [24], the European Medicines Agency [25] and the U.S. Food and Drug Administration [26, 27] have all proposed definitions with different criteria and interpretations. The different types of UTI are perhaps more aptly described as having family resemblances, a concept first described by early 20th century German philosopher Ludwig Wittgenstein in his book *Philosophical Investigations*. He argues that categories and concepts are not defined by a single set of essential characteristics but rather by a network of overlapping similarities among various members within a category, e.g. like a family sharing traits. While a single definition is not always required in clinical practice, it is crucial in research. The absence of a research definition, also referred to as a reference standard, introduces bias into estimates of diagnostic accuracy and efficacy, affecting the internal validity of a study. [28] Additionally, if different definitions are used across studies, results cannot be readily compared, compromising the external validity of a study.

Pathophysiology: host versus pathogen

Uropathogenic *Escherichia coli* (UPEC) is by far the most common pathogen causing UTI. [29] Other pathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus saprophyticus*. Enterococci and group B streptococci are frequently isolated from midstream urine, but rarely from urine obtained through single in-out catheterisation, suggesting that these pathogens do not typically cause UTI. [30]

Infection of the bladder mostly occurs through an exogenous route. Uropathogens residing in the gut first colonise the (peri)urethra and subsequently migrate into the bladder. Most research on understanding host-pathogen interactions in UTI has concentrated on infections caused by UPEC. [29] To invade the bladder,

UPEC uses adhesins located on the tip of fimbriae or on the bacterial surface to attach to uroplakins and integrins that coat the most outer layer of the urothelium, i.e. umbrella cells. Recognition of lipopolysaccharide (present on the outer membrane of Gram-negative bacteria) through Toll-like receptors on umbrella cells induces a rapid innate immune response via transcription of pro-inflammatory cytokines and chemokines. While UPEC in the bladder lumen is targeted by recruited neutrophils, antimicrobial peptides and iron-sequestering proteins, internalised UPEC is able to subvert host defences and form intracellular bacterial communities (IBCs) through multiplication. These IBCs are able to survive in the bladder environment due to additional virulence factors such as the toxin α-haemolysin, expediting nutrient acquisition via host cell lysis, and siderophores which facilitate iron uptake. Upon exfoliation of the urothelium due to inflammation and α-haemolysin, bacteria can disperse and invade neighbouring cells. Exfoliation also exposes deeper layers of the bladder epithelium where UPEC can establish quiescent intracellular reservoirs (QIRs), which can remain viable for months and may contribute to recurrences. [29] Although the pathophysiological basis for recurrent UTI in humans remains poorly understood, recent murine studies show that UTI leads to differential bladder tissue remodelling, depending on disease outcome, that affects susceptibility to subsequent UTI episodes. [31, 32] Less is known about the role of adaptive immunity in UTI. Mouse models of bladder infection show that although an adaptive immune response develops, the bacterial burden is only marginally reduced and UTI frequently recurs. [33] Secretory IgA can inhibit adhesion of UPEC to epithelial cells, and in children with acute cystitis, secretory IgA levels in urine are elevated. [34] Recently, sublingual vaccination with a suspension of whole-cell heat-inactivated *E. coli, Klebsiella pneumoniae, Proteus vulgaris,* and *Enterococcus faecalis* has shown remarkable efficacy in a placebo-controlled trial including pre- and postmenopausal women with recurrent UTI. [35] While the exact mechanism of action has not been fully elucidated, both enhanced innate and adaptive (cellular and humoral) immunity seem to play a role. [36]

Currently used diagnostics

The diagnostic approach of UTI differs per clinical presentation (typical or atypical lower urinary tract symptoms, presence of systemic signs), setting (primary care, outpatient clinic, emergency department), population (age group, biological sex, underlying risk factors) and country. Some clinicians do not perform additional

testing in women with classic lower urinary tract symptoms and absence of systemic signs, as the a priori probability of acute cystitis is high. [37] However, lower urinary tract symptoms may be caused by other conditions such as urethritis (*Chlamydia trachomatis, Neisseria gonorrhoeae*) and vaginitis (*Candida* spp., *Trichomonas vaginalis*). As such, additional testing for the presence of pyuria (i.e. leukocyturia) and bacteriuria is often performed to support the diagnosis of UTI.

In clinical practice, particularly in primary care, a urine dipstick is usually applied first, to screen for pyuria and bacteriuria. Among other analytes, the urine dipstick provides semi-quantitative results of leukocyte esterase (an enzyme produced by leukocytes in the urine) and indicates the presence or absence of nitrites. While a urine dipstick is inexpensive, easy to use and provides quick results, there are important drawbacks to note. Both leukocyte esterase and nitrite results may be false-negative due to the presence of other substances such as vitamin C. [38] Moreover, not all pathogens reduce urinary nitrates to nitrites (e.g. *Staphylococcus saprophyticus, Pseudomonas aeruginosa, Enterococcus* spp. do not). Furthermore, leukocyte esterase results correlate poorly with absolute degrees of pyuria. [39] Reported diagnostic accuracy estimates of leukocyte esterase and nitrites for diagnosing UTI vary greatly and are primarily determined by the population under investigation and the reference standard that is applied. In general, nitrites are less sensitive than specific, and leukocyte esterase is more sensitive than specific for UTI. [40-42]

Pyuria can be quantified in different ways. In the late 1960s Mabeck et al. [43] found that a leukocyte excretion rate of 400,000 per hour could distinguish UTI from asymptomatic women. This rate corresponds with a cut-off value of 10 leukocytes/mm^3 in unspun urine. [44] Nowadays, most hospitals quantify pyuria by direct or automated microscopy of (un)spun urine, generally after initial dipstick screening. Automated microscopy reduces variability in centrifugation and resuspension of urine and is more efficient than direct microscopy. [45] In recent years, an increasing number of laboratories are adopting urine flow cytometry for quantification of pyuria. Urine flow cytometers classify and quantify cells in the urine by analysing scattered light emitted by cells passing through a laser beam. While automated microscopy and urine flow cytometry have relatively short turnaround times and a high capacity, they are costly and not available to every clinician. Moreover, reference values for 'significant' pyuria vary in the literature and depend on preanalytic steps in the laboratory, quantification methods and the

studied population. Finally, pyuria may be caused by conditions other than UTI, including interstitial nephritis, urolithiasis and urological malignancies.

Bacteriuria can be determined by spreading the urine onto a plate containing a culture medium and incubating it under various conditions. This approach allows for pathogen identification and quantification, which usually takes 18-30 hours, and antimicrobial susceptibility testing, which may take another 24-48 hours, depending on the use of manual or automated methods. [46] Despite providing valuable information, long turnaround times are an important drawback of urine cultures. Additionally, there is an ongoing debate about the optimal threshold for significant bacteriuria (expressed in colony-forming units (CFU)/mL). While the traditional cut-off value of 10^5 CFU/mL is still applied by some laboratories to avoid misclassification of contamination as UTI, several studies have shown that colony-counts as low as 10^2 CFU/mL in midstream urine are indicative of true bladder bacteriuria (determined by suprapubic aspiration or single in-out catheterisation), at least in symptomatic women with *E. coli* as the causative pathogen. [30, 47] Be that as it may, urine cultures merely indicate bacteriuria, which does not necessarily equate to UTI.

Challenges in older women

In older women, diagnosing UTI presents specific challenges for several reasons. Firstly, symptom assessment is hampered by a higher prevalence of cognitive impairment and indwelling catheters in the older population. The global prevalence of dementia was estimated to be 57.4 million cases in 2019, with a female-to-male ratio of 1.69, and a predicted increase to 152.8 million cases in 2050, mainly driven by population ageing and population growth. [48] Secondly, chronic lower urinary tract symptoms, such as urgency, frequency and urinary incontinence, are common in older women and are difficult to distinguish from non-infectious causes, such as genitourinary syndrome of menopause, and overactive bladder. [49] Most importantly, 20% of community-dwelling and 50% of institutionalised older women have asymptomatic bacteriuria (ASB), defined as the presence of one or more uropathogens ≥ 10^5 CFU/mL in the absence of signs or symptoms attributable to UTI. [50-52] While the pathophysiological basis of ASB has not been completely elucidated, it is thought to arise from an interplay between host factors (e.g. reduced Toll-like receptor 4 expression [53]) and pathogen-specific factors (e.g. reduced adhesive capability of certain *E. coli* strains [54]).

Over 90% of older women with ASB have concomitant pyuria [55]. Consequently, the specificity of both pyuria and bacteriuria for UTI is low in this population, and it can be difficult to distinguish UTI from ASB with current urine diagnostics. As such, inappropriate antimicrobial treatment of asymptomatic pyuria and bacteriuria is very common. Gupta et al. [56] showed that 25% of patients with asymptomatic pyuria on routine preoperative urinalysis (without urine cultures) were treated with antimicrobials, and that the degree of pyuria predicted prescribing of antimicrobials. Moreover, in a study performed in long-term care facility residents with advanced dementia, only 19% of suspected 'UTI' episodes that were treated with antimicrobials fulfilled minimum symptom criteria (suggesting most episodes were actually ASB). [57] In older patients with cognitive impairment, who have difficulty communicating their symptoms, it may be tempting for clinicians to ascribe non-specific symptoms such as confusion or falls to a UTI, especially in the presence of pyuria and bacteriuria. However, the evidence is growing that these non-specific symptoms do not reliably predict actual UTI. [58, 59] More likely, these symptoms indicate normal fluctuations in behaviour or have other causes, such as dehydration and drug-related side effects. Although antimicrobial treatment of ASB may result in short-term microbiological cure, it does not improve survival, nor influence the frequency of subsequent UTI episodes or chronic urinary incontinence in older women. [60-63] In fact, antimicrobial treatment of ASB can lead to adverse drug reactions, interactions and toxicity, which is particularly relevant in a population with high rates of polypharmacy. [62] Moreover, antimicrobial treatment confers an eightfold increased risk of developing *Clostridioides difficile* associated diarrhoea, and it leads to subsequent isolation of multidrug resistant organisms (MDROs) from the urine. [64, 65] Besides being judicious about urine testing in older women with ambiguous symptoms, new diagnostic modalities with the ability to distinguish UTI from ASB are urgently required.

Treatment and prophylaxis in an era of antimicrobial resistance

UTI is typically treated with a course of antimicrobials. The selection of an antimicrobial regimen is primarily dependent on the site of infection, i.e. whether an agent with tissue penetration is required. First-line oral antimicrobials for empirical treatment of UTI without systemic involvement (acute cystitis) include nitrofurantoin, fosfomycin, trimethoprim, and, in some countries, pivmecillinam.

The approach to empirical treatment of UTI with systemic involvement (acute pyelonephritis with or without urosepsis) generally depends on the severity of illness. While outpatients may be treated with oral ciprofloxacin or trimethoprim-sulfamethoxazole, critically ill patients are usually treated parenterally; Dutch guidelines recommend a second or third generation cephalosporin with the option of adding aminoglycosides pending culture results. [66] In patients with recurrent UTI and insufficient efficacy of behavioural modifications, such as increased hydration [67] or postcoital voiding, oral antimicrobial prophylaxis (either daily or postcoital) is often initiated. Continuous antimicrobial prophylaxis is effective in reducing recurrence rates, even in risk groups, such as patients using clean intermittent catheterisation due to urological or neurological comorbidities. [8, 68-70] Antimicrobial options include nitrofurantoin 50 mg or 100 mg daily, and trimethoprim 100 mg daily. However, the most important drawback of antimicrobial treatment and prophylaxis, already pointed out by Alexander Fleming in 1945 [71], is the development of antimicrobial resistance (AMR).

AMR is a rising threat to global health. In fact, based on predictive statistical models, an estimated 4.95 million deaths were associated with bacterial AMR in 2019 alone, with the highest burdens in resource-limited settings. [72] *E. coli* and *Klebsiella pneumoniae (K. pneumoniae)* were the two pathogens responsible for the most AMR-attributable deaths, both common causative pathogens of UTI. AMR surveillance data of 46 European countries, published by the European Centre for Disease Prevention and Control (ECDC) in 2022, showed highest resistance rates in southern and eastern regions, compared with northern and western regions. [73] For *E. coli*, 46% of countries reported ciprofloxacin resistance rates > 25% and 11% of countries reported third generation cephalosporin resistance rates > 50%. Carbapenem resistance was more frequently reported in *K. pneumoniae* than in *E. coli*; 32% of countries reported carbapenem resistance rates > 25%. Not surprisingly, surveillance data published by the World Health Organization (WHO) in 2021 showed a wide range of resistance rates for several antimicrobial classes for *E. coli* and *K. pneumoniae*, with low and middle income countries being disproportionally affected. [74] To support antimicrobial stewardship efforts and to identify antimicrobials with the highest priorities for surveillance of use, the WHO created the Access, Watch, Reserve (AWaRe) classification, in which antimicrobials are categorised into three groups based on the potential to induce and propagate resistance. [75] Multiple agents commonly used in the treatment of UTI (e.g. ciprofloxacin and ceftriaxone) are included in the 'Watch-group' due to

increasing resistance rates. Nosocomial UTI is not infrequently caused by one of the ESKAPE pathogens (i.e. *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.), which are often difficult to treat due to their resistance to multiple classes of antimicrobials. [76] Finally, as the high incidence and recurrence rates of UTI lead to significant antimicrobial consumption, UTI is an important driver of the silent epidemic that is AMR. It is evident that alternative strategies for the treatment and prevention of UTI need to be explored to reduce the global burden of AMR. In a randomised trial evaluating different management approaches in women presenting to the primary care office with suspected acute cystitis, symptom duration and severity were similar with delayed antimicrobial therapy compared with immediate antimicrobial therapy. [77] In another randomised trial comparing ibuprofen with fosfomycin (with respective placebo dummies in both groups) in women with acute cystitis, two thirds of women in the ibuprofen group recovered without any antibiotics, albeit with a somewhat higher overall symptom burden in the ibuprofen group. [12] Besides behavioural modifications, other non-antimicrobial prophylactic strategies for recurrent UTI include vaginal oestrogen for postmenopausal women, and methenamine hippurate. [78, 79] In one open-label trial showing non-inferiority of methenamine hippurate to oral antimicrobial prophylaxis, the proportion of participants demonstrating resistance to at least one antimicrobial in *E. coli* isolated from perineal swabs was higher in the oral antimicrobial prophylaxis group at 6-12 months. [79] As the gut is a known reservoir for uropathogenic bacteria, Worby et al. [80] collected monthly faecal samples of women with recurrent UTI and controls for metagenomic analysis, and found significantly lower gut microbial richness in women with recurrent UTI. Given that some studies have shown decreased gut microbial richness in patients with intestinal colonisation of MDRO, 'gut sparing' or 'gut restorative' interventions have the potential to reduce the frequency of UTI recurrences and decrease intestinal MDRO colonisation. However, efficacy data for these alternative modalities are sparse.

Outline of the thesis

The overall aim of this thesis is to address unmet needs in the definitions, diagnosis and treatment strategies in UTI, which can contribute to improving health outcomes for individual patients and reducing antimicrobial resistance. This thesis comprises three parts. The first part focuses on the definition of UTI

in research. To evaluate the heterogeneity of UTI definitions in recent studies, a systematic review was performed, which is described in **Chapter 2**. Given the high heterogeneity of study definitions and conflicting research guidelines, a Delphi consensus study involving an international, multidisciplinary panel of UTI experts was conducted to construct a reference standard for UTI research. This consensus study is reported in **Chapter 3**.

The second part of this thesis centres on diagnostic challenges of UTI in older women. **Chapter 4** describes a case-control study including older women with UTI and ASB, in which the diagnostic accuracy of two pyuria quantification methods (automated microscopy and urine flow cytometry) for UTI is determined, and an optimal pyuria threshold for older women is sought. Due to the limitations of current urine diagnostics in older women, the diagnostic accuracy of twelve novel urine biomarkers is evaluated in the same study population, which is reported in **Chapter 5**.

In the third and last part of this thesis alternative strategies for the treatment and prevention of UTI are explored. As stated in the previous paragraph, (systemic) antimicrobial prophylaxis and treatment have important drawbacks. Therefore, we performed a cohort study, described in **Chapter 6**, to assess the treatment satisfaction, long-term safety, and efficacy of a non-systemic antimicrobial prophylactic strategy, i.e. intravesical aminoglycoside instillations. As intestinal MDRO colonisation may precede invasive infection and facilitates spread within communities and hospitals, the efficacy of faecal microbiota transplantation for MDRO decolonisation was assessed in a systematic review in **Chapter 7**.

Chapter 8 provides a summary and a discussion of the results, resulting in a conclusion and views on possible further research.

References

1. Zeng Z, Zhan J, Zhang K, Chen H, Cheng S. Global, regional, and national burden of urinary tract infections from 1990 to 2019: an analysis of the global burden of disease study 2019. World J Urol **2022**; 40(3): 755-63.
2. Schappert SM, Rechtsteiner EA. Ambulatory medical care utilization estimates for 2007. Vital Health Stat 13 **2011**; (169): 1-38.
3. Cijfers huisartsen - Gezondheidsproblemen. Available at: https://www.nivel.nl/nl/nivel-zorgregistraties-eerste-lijn/cijfers-zorg-huisarts-huisartsenpost-paramedici/cijfers-zorg-huisartsen/gezondheidsproblemen. Accessed 1st of July 2023.
4. Hooton TM, Scholes D, Hughes JP, et al. A prospective study of risk factors for symptomatic urinary tract infection in young women. N Engl J Med **1996**; 335(7): 468-74.
5. Ahmed H, Farewell D, Jones HM, Francis NA, Paranjothy S, Butler CC. Incidence and antibiotic prescribing for clinically diagnosed urinary tract infection in older adults in UK primary care, 2004-2014. PLoS One **2018**; 13(1): e0190521.
6. Caljouw MA, den Elzen WP, Cools HJ, Gussekloo J. Predictive factors of urinary tract infections among the oldest old in the general population. A population-based prospective follow-up study. BMC Med **2011**; 9: 57.
7. Ikaheimo R, Siitonen A, Heiskanen T, et al. Recurrence of urinary tract infection in a primary care setting: analysis of a 1-year follow-up of 179 women. Clin Infect Dis **1996**; 22(1): 91-9.
8. Beerepoot MA, ter Riet G, Nys S, et al. Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women. Arch Intern Med **2012**; 172(9): 704-12.
9. Thompson J, Marijam A, Mitrani-Gold FS, Wright J, Joshi AV. Activity impairment, health-related quality of life, productivity, and self-reported resource use and associated costs of uncomplicated urinary tract infection among women in the United States. PLoS One **2023**; 18(2): e0277728.
10. Ennis SS, Guo H, Raman L, Tambyah PA, Chen SL, Tiong HY. Premenopausal women with recurrent urinary tract infections have lower quality of life. Int J Urol **2018**; 25(7): 684-9.
11. Curns AT, Holman RC, Sejvar JJ, Owings MF, Schonberger LB. Infectious disease hospitalizations among older adults in the United States from 1990 through 2002. Arch Intern Med **2005**; 165(21): 2514-20.
12. Gagyor I, Bleidorn J, Kochen MM, Schmiemann G, Wegscheider K, Hummers-Pradier E. Ibuprofen versus fosfomycin for uncomplicated urinary tract infection in women: randomised controlled trial. BMJ **2015**; 351: h6544.
13. Jansaker F, Li X, Vik I, Frimodt-Moller N, Knudsen JD, Sundquist K. The Risk of Pyelonephritis Following Uncomplicated Cystitis: A Nationwide Primary Healthcare Study. Antibiotics (Basel) **2022**; 11(12).
14. Mosharafa AA, Torky MH, El Said WM, Meshref A. Rising incidence of acute prostatitis following prostate biopsy: fluoroquinolone resistance and exposure is a significant risk factor. Urology **2011**; 78(3): 511-4.

15. Lafaurie M, Chevret S, Fontaine JP, et al. Antimicrobial for 7 or 14 Days for Febrile Urinary Tract Infection in Men: A Multicenter Noninferiority Double-Blind, Placebo-Controlled, Randomized Clinical Trial. Clin Infect Dis **2023**; 76(12): 2154-62.

16. Su ZT, Zenilman JM, Sfanos KS, Herati AS. Management of Chronic Bacterial Prostatitis. Curr Urol Rep **2020**; 21(7): 29.

17. Czaja CA, Scholes D, Hooton TM, Stamm WE. Population-based epidemiologic analysis of acute pyelonephritis. Clin Infect Dis **2007**; 45(3): 273-80.

18. Johnson JR, Russo TA. Acute Pyelonephritis in Adults. N Engl J Med **2018**; 378(1): 48-59.

19. Kim Y, Seo MR, Kim SJ, et al. Usefulness of Blood Cultures and Radiologic Imaging Studies in the Management of Patients with Community-Acquired Acute Pyelonephritis. Infect Chemother **2017**; 49(1): 22-30.

20. van Nieuwkoop C, van der Starre WE, Stalenhoef JE, et al. Treatment duration of febrile urinary tract infection: a pragmatic randomized, double-blind, placebo-controlled non-inferiority trial in men and women. BMC Med **2017**; 15(1): 70.

21. Esparcia A, Artero A, Eiros JM, et al. Influence of inadequate antimicrobial therapy on prognosis in elderly patients with severe urinary tract infections. Eur J Intern Med **2014**; 25(6): 523-7.

22. Tal S, Guller V, Levi S, et al. Profile and prognosis of febrile elderly patients with bacteremic urinary tract infection. J Infect **2005**; 50(4): 296-305.

23. Levy MM, Artigas A, Phillips GS, et al. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. Lancet Infect Dis **2012**; 12(12): 919-24.

24. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) Events. Available at: https://www.cdc.gov/nhsn/psc/uti/. Accessed 8th of June.

25. Evaluation of medicinal products indicated for treatment of bacterial infections. Available at: https://www.ema.europa.eu/en/evaluation-medicinal-products-indicated-treatment-bacterial-infections Accessed 8th of June.

26. Uncomplicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/uncomplicated-urinary-tract-infections-developing-drugs-treatment-guidance-industry Accessed 8th of June.

27. Complicated Urinary Tract Infections: Developing Drugs for Treatment. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/complicated-urinary-tract-infections-developing-drugs-treatment Accessed 8th of June.

28. de Groot JA, Bossuyt PM, Reitsma JB, et al. Verification problems in diagnostic accuracy studies: consequences and solutions. BMJ **2011**; 343: d4770.

29. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol **2015**; 13(5): 269-84.

30. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med **2013**; 369(20): 1883-91.

31. Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA, Hultgren SJ. Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic Escherichia coli bladder infection. FEMS Microbiol Rev **2012**; 36(3): 616-48.

32. Russell SK, Harrison JK, Olson BS, et al. Uropathogenic Escherichia coli infection-induced epithelial trained immunity impacts urinary tract disease outcome. Nat Microbiol **2023**; 8(5): 875-88.

33. Lacerda Mariano L, Ingersoll MA. The immune response to infection in the bladder. Nat Rev Urol **2020**; 17(8): 439-58.

34. Butler D, Ambite I, Wan MLY, Tran TH, Wullt B, Svanborg C. Immunomodulation therapy offers new molecular strategies to treat UTI. Nat Rev Urol **2022**; 19(7): 419-37.

35. Lorenzo-Gómez M-F, Foley S, Nickel JC, et al. Sublingual MV140 for Prevention of Recurrent Urinary Tract Infections. NEJM Evidence **2022**; 1(4): EVIDoa2100018.

36. Martin-Cruz L, Sevilla-Ortega C, Benito-Villalvilla C, et al. A Combination of Polybacterial MV140 and Candida albicans V132 as a Potential Novel Trained Immunity-Based Vaccine for Genitourinary Tract Infections. Front Immunol **2020**; 11: 612269.

37. Bent S, Nallamothu BK, Simel DL, Fihn SD, Saint S. Does this woman have an acute uncomplicated urinary tract infection? JAMA **2002**; 287(20): 2701-10.

38. Ko DH, Jeong TD, Kim S, et al. Influence of Vitamin C on Urine Dipstick Test Results. Ann Clin Lab Sci **2015**; 45(4): 391-5.

39. van den Broek D, Keularts IM, Wielders JP, Kraaijenhagen RJ. Benefits of the iQ200 automated urine microscopy analyser in routine urinalysis. Clin Chem Lab Med **2008**; 46(11): 1635-40.

40. Timmermans AE, Walter AEGM, Van Duijn NP, Timmerman CP. De diagnostische waarde van urineonderzoek in de huisartspraktijk. Huisarts Wet **1996**; 39(4): 165-8.

41. Christiaens TCM, De Meyere M, Derese A. Disappointing specificity of the leucocyte-esterase test for the diagnosis of urinary tract infection in general practice. Eur J Gen Pract **1998**; 4: 144-8.

42. Hooton TM, Roberts PL, Stapleton AE. Asymptomatic Bacteriuria and Pyuria in Premenopausal Women. Clin Infect Dis **2021**; 72(8): 1332-8.

43. Mabeck CE. Studies in urinary tract infections. IV. Urinary leucocyte excretion in bacteriuria. Acta Med Scand **1969**; 186(3): 193-8.

44. Stamm WE. Measurement of pyuria and its relation to bacteriuria. Am J Med **1983**; 75(1B): 53-8.

45. Oyaert M, Delanghe J. Progress in Automated Urinalysis. Ann Lab Med **2019**; 39(1): 15-22.

46. Davenport M, Mach KE, Shortliffe LMD, Banaei N, Wang TH, Liao JC. New and developing diagnostic technologies for urinary tract infections. Nat Rev Urol **2017**; 14(5): 296-310.

47. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med **1982**; 307(8): 463-8.

48. Collaborators GBDDF. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health **2022**; 7(2): e105-e25.

49. Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol **2006**; 50(6): 1306-14; discussion 14-5.

50. Nicolle LE, Gupta K, Bradley SF, et al. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis **2019**; 68(10): 1611-5.

51. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis **2008**; 40(10): 804-10.

52. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc **1995**; 43(7): 772-5.

53. Ragnarsdottir B, Samuelsson M, Gustafsson MC, Leijonhufvud I, Karpman D, Svanborg C. Reduced toll-like receptor 4 expression in children with asymptomatic bacteriuria. J Infect Dis **2007**; 196(3): 475-84.

54. Roos V, Schembri MA, Ulett GC, Klemm P. Asymptomatic bacteriuria Escherichia coli strain 83972 carries mutations in the foc locus and is unable to express F1C fimbriae. Microbiology (Reading) **2006**; 152(Pt 6): 1799-806.

55. Boscia JA, Abrutyn E, Levison ME, Pitsakis PG, Kaye D. Pyuria and asymptomatic bacteriuria in elderly ambulatory women. Ann Intern Med **1989**; 110(5): 404-5.

56. Gupta K, O'Brien W, Gallegos-Salazar J, Strymish J, Branch-Elliman W. How Testing Drives Treatment in Asymptomatic Patients: Level of Pyuria Directly Predicts Probability of Antimicrobial Prescribing. Clin Infect Dis **2020**; 71(3): 614-21.

57. Mitchell SL, Shaffer ML, Loeb MB, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med **2014**; 174(10): 1660-7.

58. Sundvall PD, Elm M, Ulleryd P, et al. Interleukin-6 concentrations in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly: a cross sectional study of 421 nursing home residents. BMC Geriatr **2014**; 14: 88.

59. Sundvall PD, Ulleryd P, Gunnarsson RK. Urine culture doubtful in determining etiology of diffuse symptoms among elderly individuals: a cross-sectional study of 32 nursing homes. BMC Fam Pract **2011**; 12: 36.

60. Abrutyn E, Mossey J, Berlin JA, et al. Does asymptomatic bacteriuria predict mortality and does antimicrobial treatment reduce mortality in elderly ambulatory women? Ann Intern Med **1994**; 120(10): 827-33.

61. Boscia JA, Kobasa WD, Knight RA, Abrutyn E, Levison ME, Kaye D. Therapy vs no therapy for bacteriuria in elderly ambulatory nonhospitalized women. JAMA **1987**; 257(8): 1067-71.

62. Nicolle LE, Mayhew WJ, Bryan L. Prospective randomized comparison of therapy and no therapy for asymptomatic bacteriuria in institutionalized elderly women. Am J Med **1987**; 83(1): 27-33.

63. Ouslander JG, Schapira M, Schnelle JF, et al. Does eradicating bacteriuria affect the severity of chronic urinary incontinence in nursing home residents? Ann Intern Med **1995**; 122(10): 749-54.

64. Das R, Towle V, Van Ness PH, Juthani-Mehta M. Adverse outcomes in nursing home residents with increased episodes of observed bacteriuria. Infect Control Hosp Epidemiol **2011**; 32(1): 84-6.

65. Rotjanapan P, Dosa D, Thomas KS. Potentially inappropriate treatment of urinary tract infections in two Rhode Island nursing homes. Arch Intern Med **2011**; 171(5): 438-43.

66. Optimization of the antibiotic policy in the Netherlands: SWAB guidelines for antimicrobial therapy of urinary tract infections in adults Available at: https://swab.nl/nl/urineweginfecties. Accessed 21st of November 2023.

67. Hooton TM, Vecchio M, Iroz A, et al. Effect of Increased Daily Water Intake in Premenopausal Women With Recurrent Urinary Tract Infections: A Randomized Clinical Trial. JAMA Intern Med **2018**; 178(11): 1509-15.

68. Beerepoot MA, ter Riet G, Nys S, et al. Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. Arch Intern Med **2011**; 171(14): 1270-8.

69. Albert X, Huertas I, Pereiro, II, Sanfelix J, Gosalbes V, Perrota C. Antibiotics for preventing recurrent urinary tract infection in non-pregnant women. Cochrane Database Syst Rev **2004**; 2004(3): CD001209.

70. Fisher H, Oluboyede Y, Chadwick T, et al. Continuous low-dose antibiotic prophylaxis for adults with repeated urinary tract infections (AnTIC): a randomised, open-label trial. Lancet Infect Dis **2018**; 18(9): 957-68.

71. Rosenblatt-Farrell N. The landscape of antibiotic resistance. Environ Health Perspect **2009**; 117(6): A244-50.

72. Antimicrobial Resistance C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet **2022**; 399(10325): 629-55.

73. Surveillance of antimicrobial resistance in Europe. Available at: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2022-data. Accessed 21st of November 2023.

74. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report. Available at: https://www.who.int/publications/i/item/9789240027336. Accessed 21st of November 2023.

75. WHO Access, Watch, Reserve (AWaRe) classification of antibiotics for evaluation and monitoring of use, 2021. Geneva: World Health Organization; 2021 (WHO/MHP/HPS/EML/2021.04). Available at: https://www.who.int/publications/i/item/2021-aware-classification. Accessed 25th of November 2023.

76. De Oliveira DMP, Forde BM, Kidd TJ, et al. Antimicrobial Resistance in ESKAPE Pathogens. Clin Microbiol Rev **2020**; 33(3).

77. Little P, Moore MV, Turner S, et al. Effectiveness of five different approaches in management of urinary tract infection: randomised controlled trial. BMJ **2010**; 340: c199.

78. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med **1993**; 329(11): 753-6.

79. Harding C, Mossop H, Homer T, et al. Alternative to prophylactic antibiotics for the treatment of recurrent urinary tract infections in women: multicentre, open label, randomised, non-inferiority trial. BMJ **2022**; 376: e068229.

80. Worby CJ, Schreiber HLt, Straub TJ, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol **2022**; 7(5): 630-9.

Part I

Defining UTI

Chapter 2

Definitions of urinary tract infection in current research: a systematic review

Manu P. Bilsen*, Rosa M.H. Jongeneel*, Caroline Schneeberger, Tamara N. Platteel, Cees van Nieuwkoop, Lona Mody, Jeffrey M. Caterino, Suzanne E. Geerlings, Béla Köves, Florian Wagenlehner, Simon P. Conroy, Leo G. Visser, Merel M.C. Lambregts

*these authors contributed equally to this manuscript

Open Forum Infect Dis. 2023 Jun 27;10(7):ofad332

Abstract

Defining urinary tract infection (UTI) is complex, as numerous clinical and diagnostic parameters are involved. In this systematic review we aimed to gain insight into how UTI is defined across current studies. We included 47 studies, published between January 2019 and May 2022, investigating therapeutic or prophylactic interventions in adult patients with UTI. Signs and symptoms, pyuria and a positive urine culture were required in 85%, 28% and 55% of study definitions, respectively. Five studies (11%) required all three categories for the diagnosis of UTI. Thresholds for significant bacteriuria varied from 10^3 to 10^5 colony-forming units/mL. None of the 12 studies including acute cystitis and 2/12 (17%) defining acute pyelonephritis used identical definitions. Complicated UTI was defined by both host factors and systemic involvement in 9/14 (64%) studies. In conclusion, UTI definitions are heterogeneous across recent studies, highlighting the need for a consensus-based, research reference standard for UTI.

Introduction

Urinary tract infection (UTI) refers to a plethora of clinical phenotypes, including cystitis, pyelonephritis, prostatitis, urosepsis, and catheter-associated UTI (CA-UTI). [1, 2] In both clinical practice and in research, the diagnosis of UTI is based on a multitude of clinical signs and symptoms and diagnostic tests. Signs and symptoms can be further subdivided into (1) lower urinary tract symptoms (LUTS), such as dysuria, frequency, and urgency, (2) systemic signs and symptoms, such as fever, and (3) non-specific signs and symptoms, such as nausea and malaise. Commonly used diagnostic tests include urine dipstick for determining the presence of leukocyte esterase and nitrites, microscopy or flowcytometry for quantification of pyuria, and urine and blood cultures.

When defining and diagnosing UTI, numerous combinations of signs, symptoms and outcomes of diagnostic tests are possible, and this diversity is reflected in various research guidelines. For drug development and approval purposes, the European Medicines Agency (EMA) [3] and Food and Drug Administration (FDA) [4, 5] have developed guidelines for clinical trials evaluating antimicrobials for the treatment of UTI, summarised in **Table 1**. These guidelines provide definitions for uncomplicated UTI, complicated UTI, and acute pyelonephritis. McGeer et al. [6] have developed research guidelines for studies in long-term care facilities (LTCF). Clinical practice guidelines include the Infectious Diseases Society of America (currently being updated) [7] and European Association of Urology guidelines [8]. It is important to distinguish between research guidelines and clinical practice guidelines as the latter are meant for treatment recommendations, and the definitions in these clinical guidelines are generally based on often-limited diagnostic information available when assessing a patient in the clinical, near-patient setting.

While the aforementioned research guidelines overlap in the sense that they all include a combination of symptoms and evidence of pyuria and/or bacteriuria in the definition of UTI, they also differ. For instance, none of these guidelines include the same set (or minimum number) of symptoms for the diagnosis of UTI. Moreover, the definition of complicated UTI is variable, and either based on systemic signs and symptoms or the presence of host factors predisposing the patient to a complicated clinical course (e.g. functional or anatomical abnormalities of the urinary tract).

Table 1: EMA and FDA definitions of uncomplicated and complicated UTI

Category	EMA – uUTI	FDA – uUTI	EMA – cUTI	FDA – cUTI
Symptoms	A minimum number of symptoms, such as frequency, urgency, and dysuria.	≥ 2: dysuria, frequency, urgency, suprapubic pain (note: lower abdominal discomfort is also mentioned in another section of the guidance document) Patients should **not** have signs or symptoms of systemic illness such as fever > 38°C, shaking chills or other manifestations suggestive of cUTI	A minimum number of signs/ symptoms compatible with an ongoing process in the urinary tract, such as flank or pelvic pain, CVA tenderness, dysuria, frequency or urgency	≥ 2: chills or rigors or warmth associated with fever (>38°C), flank or pelvic pain, dysuria or frequency or urgency, CVA tenderness (note: malaise is also mentioned in another section of the guidance document)
Host factors	Female patients	Female patients with normal anatomy of the urinary tract	≥ 1: indwelling catheter, urinary retention, obstruction, neurogenic bladder. AP is mentioned separately from cUTI, but it is not further defined	≥ 1: indwelling urinary catheter, neurogenic bladder, obstructive uropathy, azotemia caused by intrinsic renal disease, urinary retention (including retention caused by BPH). AP is a subset of cUTI regardless of underlying abnormalities of the urinary tract
Pyuria	> 10 leukocytes/ mm^3	'A microscopic evaluation for pyuria or dipstick analysis for leukocytes, nitrites or a catalase test should be performed'	> 10 leukocytes/ mm^3	Urine dipstick positive for leukocyte esterase **or** > 10 leukocytes/ mm^3
Bacteriuria	> 10^5 CFU/mL of a single relevant pathogen	≥ 10^5 CFU/mL of a single species of bacteria	> 10^5 CFU/mL of a single, or no more than two relevant pathogens	≥ 10^5 CFU/mL of a single species of bacteria

In the EMA guidelines bacteriuria definitions were mentioned in the description of the microbiological intention-to-treat population. In the FDA guidelines, they were also mentioned separately, under clinical microbiology considerations. Abbreviations: EMA = European Medicines Agency, FDA = Food and Drug Administration, uUTI = uncomplicated UTI, cUTI = complicated UTI, CVA = costovertebral angle, AP = acute pyelonephritis, CFU = colony-forming units

It is probable that this wide range of possible definitions and different research guidelines pose problems for researchers conducting studies with patients with UTI. A uniform research definition increases homogeneity between studies, which is important for the interpretation, synthesis and comparability of results, and mitigates the risk of misclassification bias. This is especially relevant in an era of rising antimicrobial resistance, in which novel antimicrobials are being investigated in large randomised controlled trials. The aim of this systematic review is to evaluate how UTI is defined in current studies, and to what extent these definitions differ between studies.

Methods

This systematic review was conducted in accordance with the *Preferred Reporting Items for Systematic reviews and Meta-analyses* (PRISMA) 2020 guidelines [9].

Eligibility criteria

Studies published between January 2019 and May 2022, investigating any therapeutic or prophylactic intervention in adults with (recurrent) UTI were eligible for inclusion. Given the fact that definitions tend to change over time, this time frame was chosen to reflect the most recent consensus. In addition, updated FDA and EMA guidelines were published in 2019. We excluded studies concerning only prostatitis, catheter-associated UTI (CA-UTI), pericatheter or perioperative prophylaxis or ASB. Studies investigating patients with spinal cord injury or neurogenic bladder were also excluded, because separate UTI definitions are mostly used for patients who are unable to experience (or have altered perception of) LUTS. Finally, we excluded systematic reviews, meta-analyses, and studies published in non-English language journals

Search strategy

Multiple electronic databases (PubMed, Embase, Web of Science and the Cochrane library) were searched on May 16th, 2022. Our search strategy was constructed by a research librarian and was based on a PICO-style approach. We applied language and publication year filters as described above and used an 'article type' type filter for clinical trials. The complete search strategy is provided in the **Supplement**.

Data extraction and analysis

Covidence software was used for screening and data extraction. References were imported and duplicates were removed. Title and abstract screening, full-text screening and data extraction were performed by two independent reviewers (M.P.B., R.M.H.J.). In case of disagreement, a third researcher was consulted (M.M.C.L.) and a final decision was based on consensus.

For each study, the following data were collected: study design, setting, population, intervention, and the type of UTI under investigation. Criteria for the definition of UTI were subdivided into three categories: signs and symptoms, urinalysis and urine culture. For each of these categories, we assessed whether they were required or conditionally required (i.e. dependent on the presence of other categories) for the diagnosis of UTI. If categories were not mentioned, or if they were only required for a secondary outcome or definition, they were considered as not required. Definitions were derived from eligibility criteria, unless definitions were explicitly stated elsewhere. For signs and symptoms, additional data were collected on minimum number of symptoms and symptom specification (e.g. if fever and frequency were further defined). Moreover, we recorded which symptoms were part of the definition of acute cystitis, acute pyelonephritis and UTI if a clinical phenotype was not mentioned (henceforth described as 'UTI – phenotype not specified'). For the urinalysis category, we extracted which methods were used for determining pyuria, which cut-off values were applied, and whether nitrites were part of the UTI definition. Regarding the urine culture category, we recorded the cut-off value for CFU/mL and the maximum number of uropathogens. For all three categories, we assessed whether study definitions met FDA and EMA guideline requirements. Concerning complicated UTI, we collected the same components of the definition as described above, but we also assessed whether the definition was based on host factors, systemic involvement, or a combination of both. Finally, we compared definitions between studies, stratified per UTI type. No risk of bias assessment was performed as we studied definitions instead of outcomes. Data are summarised as proportions.

Results

Study selection and study characteristics

The study selection process is summarised in a PRISMA flowchart (**Figure 1**). We screened 348 reports published between January 2019 and May 2022. Studies that were excluded during title and abstract screening (n = 290) mainly involved

patients with CA-UTI or conditions other than UTI (e.g. interstitial cystitis), or investigated pericatheter or perioperative prophylaxis. During full-text screening, seven non-English articles and secondary analyses of articles already included in the study using our search criteria, were excluded. A total of 47 randomised controlled trials and cohort studies with a median of 145 participants were included. [10–56]

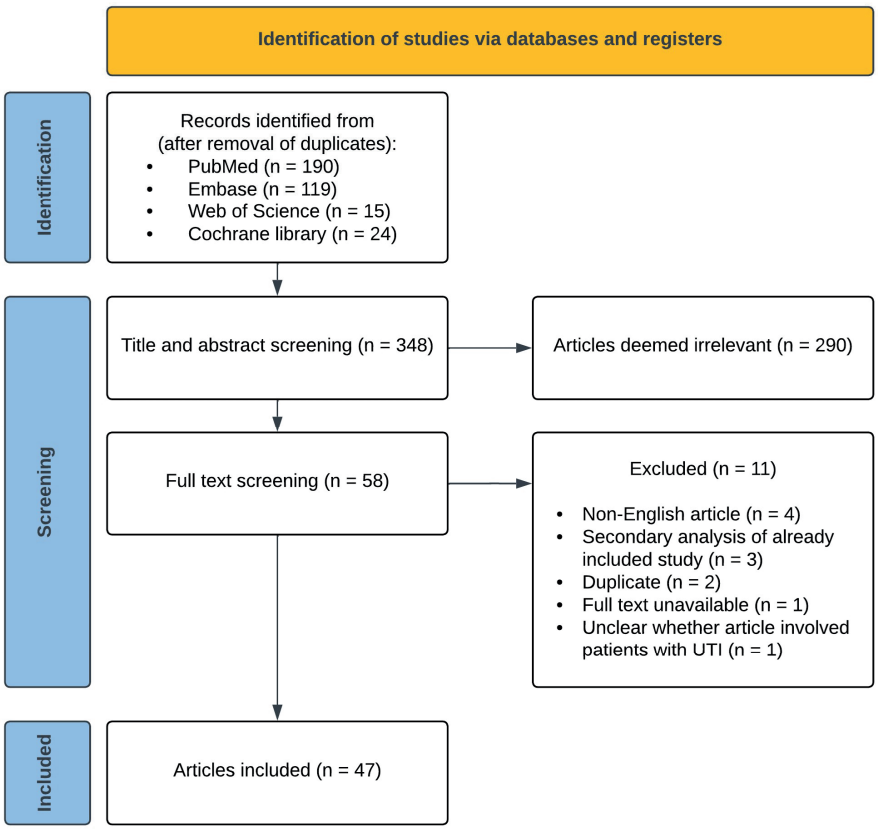


Figure 1: PRISMA flowchart of the study selection process. Abbreviations: UTI = urinary tract infection.

Thirty-one studies (66%) investigated antimicrobials for the treatment of UTI, and 15 (32%) evaluated antimicrobial prophylaxis for recurrent UTI. Sixteen studies (34%) only included women, four studies (9%) only included men, and 27 studies (57%) included both. Participants were hospitalised in 25 studies (53%)

and treated through an outpatient or primary care clinic in 22 studies (47%). None of the included studies were conducted in long-term care facilities. Twelve studies (26%) included acute cystitis, 16 (34%) included acute pyelonephritis and 13 (28%) included 'UTI – phenotype not specified'. A table containing details of all included studies is provided in **Supplementary Table 1**.

UTI definition and heterogeneity

Table 2 shows how UTI was defined across the included studies. In 11 studies (23%) the definition consisted of only signs and symptoms, in 16 studies (34%) the definition consisted of both signs and symptoms and a positive urine culture, and in 5 studies (11%) all three components (signs and symptoms, the presence of pyuria and a positive urine culture) were required for the diagnosis of UTI. None of the studies investigating acute cystitis (n = 12) or 'UTI – phenotype not specified' (n = 13) included the same set of symptoms and diagnostic criteria in their definition. Of the studies defining acute pyelonephritis, two (17%) used identical definitions.

Signs and symptoms

Signs and symptoms were required for the diagnosis of UTI in 40 studies (85%). Of these, 34 (85%) specified signs and symptoms in the definition. The different signs and symptoms that were included in the definition of acute cystitis, acute pyelonephritis and 'UTI – phenotype not specified' are highlighted in **Table 3**. FDA guidelines [4] require a minimum of two of the following symptoms for patients with uncomplicated UTI: dysuria, urgency, frequency and suprapubic pain. Two out of 12 studies (17%) met these criteria. Flank pain and/or costovertebral angle tenderness, fever, nausea and/or vomiting, and dysuria were most often included in the definition of acute pyelonephritis. Frequency was not further specified in any study. Perineal and/or prostate pain was part of the definition in 3/31 (10%) studies involving men. A specific temperature cut-off for fever was defined in 7/17 (65%) studies that included fever in the definition of UTI.

Table 2: Categories of UTI definition.

Categories of UTI definition (n = 47)	n (%)
Signs and symptoms	
Required	40 (85)
Conditionally required	1 (2)
Not required	6 (13)
Signs and symptoms specified	34/40 (85)
Minimum number of symptoms specified	24/40 (60)
Pyuria	
Required	13 (28)
Conditionally required	4 (9)
Not required	30 (64)
Method of establishing pyuria specified	14/17 (82)
Dipstick only	2 (14)
Quantification only	4 (29)
Both methods allowed	8 (57)
Cut-off for pyuria specified	12/12 (100)
> 5 leukocytes/hpf	2 (17)
> 10 leukocytes/µl or > 10 leukocytes/hpf	10 (83)
Urine culture	
Required	26 (55)
Conditionally required	1 (2)
Not required	20 (43)
Cut-off for CFU/mL specified	19/27 (70)
> 10^3 CFU/mL	8 (42)
> 10^4 CFU/mL	4 (21)
> 10^5 CFU/mL	7 (37)
Maximum number of uropathogens specified	4/27 (15)
Urine collection method specified	12/47 (26)

If categories were not mentioned, they were considered as not required. Definitions were derived from eligibility criteria, unless definitions were explicitly stated elsewhere. Percentages may not add up to 100 due to rounding. Abbreviations: UTI = urinary tract infection, hpf = high-power field, CFU = colony-forming units

Table 3: Symptoms and signs in different types of UTI.

Symptoms & signs	Acute cystitis (n = 12)	Acute pyelonephritis (n = 16)*	UTI – phenotype not specified (n = 13)
Dysuria	9 (75)	8 (50)	9 (69)
Urgency	9 (75)	6 (38)	7 (54)
Frequency	9 (75)	7 (44)	6 (46)
Suprapubic pain	5 (42)	0	6 (46)
Macroscopic haematuria	4 (33)	0	4 (31)
Lower abdominal pain	2 (17)	0	1 (8)
Perineal/prostate pain	1 (8)	0	2 (15)
Pelvic pain	0	2 (13)	1 (8)
Flank pain or CVA tenderness	1 (8)	12 (75)	2 (15)
New urinary incontinence	0	0	1 (8)
Worsening incontinence	0	0	1 (8)
Fever	0	12 (75)	2 (15)
Chills or rigors	0	7 (44)	0
Nausea or vomiting	0	8 (50)	0
Symptoms not specified	3 (25)	4 (25)	2 (15)

*This included all studies investigating acute pyelonephritis, either alone or in conjunction with other types of UTI. All symptoms and signs are shown as n (%). Other symptoms mentioned in studies focusing on acute cystitis or 'UTI – phenotype not specified' were: vesical tenesmus (n = 1), malodorous and/or cloudy urine (n = 1), hypogastric pain (n = 1), and nocturia (n = 1). Additional criteria for the definition of acute pyelonephritis not mentioned in the table: elevated serum inflammatory parameters (n = 1), signs of pyelonephritis on ultrasound or computed tomography (n = 1), and hypotension (n =1). Abbreviations: UTI = urinary tract infection, CVA = costovertebral angle.

Urinalysis and urine culture

The presence of pyuria was required for the diagnosis of UTI in 13/47 (28%) studies, while both FDA and EMA guidelines [3-5] require pyuria in their definition of UTI. A cut-off for pyuria was specified in 12 studies, of which 10 (83%) applied a cut-off value of > 10 leukocytes/µl or > 10 leukocytes/high-power field. None of the included studies required the presence of nitrites for the diagnosis of UTI, although they were conditionally required in three studies (6%). A positive urine culture was mandatory for UTI diagnosis in 26/47 (55%) studies, of which 12 (55%) were conducted in the primary care or outpatient setting and 14 (56%) involved hospitalised patients. Of the 19 studies that mentioned a cut-off value for CFU/mL, 8 (42%) used a cut-off of 10^3 CFU/mL. Out of all studies, 7 (15%) required a positive urine culture with at least 10^5 CFU/mL, complying with EMA and FDA guidelines. [3-5]

Complicated UTI

We included 14 studies that defined complicated UTI. Three (21%) based their definition on complicating host factors only, one (7%) on systemic involvement only, and nine (64%) on both host factors and systemic involvement. The various host factors included in the definition are provided in **Table 4**. Male sex was considered a complicating factor in two studies (17%).

Table 4: Definition of complicated UTI.

Complicated UTI (n = 14)	n (%)
How is complicated UTI defined?	
Both host factors and systemic involvement	9 (64)
Only host factors	3 (21)
Only systemic involvement	1 (7)
Complicated UTI not further defined	1 (7)
Which host factors are part of complicated UTI criteria?*	
Obstructive uropathy	11 (92)
Functional or anatomical abnormalities of the urinary tract	10 (83)
Indwelling catheter or nephrostomy tube	9 (75)
Intrinsic renal disease	8 (67)
Urinary retention in men due to BPH	5 (42)
Urinary retention in general	3 (25)
Male sex (regardless of urinary retention)	2 (17)
Diabetes mellitus	2 (17)
Systemic lupus erythematosus	2 (17)
Pregnancy	1 (8)
Immunocompromised state	1 (8)
Kidney transplant recipient	1 (8)

*Host factors were specified in n = 12 studies, this was used as the denominator for the proportions. For the purpose of this table, systemic involvement was defined as the presence of fever and/or rigors in the criteria for diagnosis of complicated UTI. Abbreviations: UTI = urinary tract infection, BPH = benign prostatic hyperplasia

Discussion

In this systematic review we demonstrate that UTI definitions used in current research studies are highly heterogeneous in terms of clinical signs and diagnostic tests. In addition, few studies met symptom, pyuria and urine culture criteria mentioned in existing research guidelines.

Signs and symptoms

The presence of signs and symptoms was required in the majority of UTI definitions used in the included studies. As symptoms and signs remain the cornerstone of UTI diagnosis, it is noteworthy that 15% of studies did not require signs and symptoms for the diagnosis of UTI and an even greater number of studies did not specify which symptoms and signs needed to be present. Defining specific symptoms may help to mitigate the risk of misclassification. Symptom specification is especially relevant in studies involving older patients with UTI, given the high background prevalence of asymptomatic bacteriuria and pyuria. [57–59] Most of the studies that did clarify which symptoms were part of the UTI definition included classic UTI-associated symptoms, such as dysuria, frequency and urgency. However, we also found a broad variety of non-specific manifestations, particularly in studies that did not define the UTI phenotype under investigation. Regardless of the unclear clinical relevance of non-specific symptoms in UTI, this diversity of symptoms contributes to heterogeneity between studies, which is supported by our finding that few of the included studies used the same set of symptoms to define UTI. Furthermore, in over a third of the included reports, a minimum number of symptoms (for diagnosis) was not mentioned. Given the fact that even classic LUTS are not 100% specific for UTI, and probability of UTI increases when a combination of symptoms is present, a minimum number of symptoms should be specified. [60]

Pyuria and bacteriuria

Interestingly, less than a third of included studies required the presence of pyuria in the definition of UTI. With the exception of patients with absolute neutropenia and complete obstructive uropathy, pyuria is present in virtually all symptomatic patients with bacteriuria, and its absence has a high negative predictive value for UTI. [61–63] In the included studies, pyuria was rarely quantified and thresholds for significant pyuria were low. A recent study has shown that low pyuria cut-offs should be avoided in older women, as the specificity for UTI is very low in this population. [64] Moreover, studies used different units of measurement interchangeably (i.e. identical thresholds were applied for cells per µl and hpf), while results are influenced by different (pre)analytical procedures and previous studies have shown a µl/hpf ratio of 5:1. [65] Be that as it may, quantification of pyuria in UTI studies should be encouraged, and pyuria should be included in the definition of UTI to reduce the risk of misclassification.

As growth of a uropathogen supports the diagnosis of UTI in a symptomatic patient, it is surprising that a positive urine culture was not part of the UTI definition in approximately half of the included studies. Even though urine cultures are not always required in a clinical setting (e.g. in primary care), we believe that culture confirmation should at least be encouraged in a research setting. Furthermore, we found that studies used varying cut-offs for significant bacteriuria, ranging from 10^3 to 10^5 CFU/mL, while EMA and FDA guidelines both recommend a threshold of 10^5 CFU/mL. The question remains whether this is the optimal cut-off [66]: colony-counts as low as 10^2 CFU/mL in midstream urine have been found in symptomatic premenopausal women with *E. coli* bacteriuria. [61, 62]

Complicated UTI

Studies differed widely in their definition of complicated UTI. Since the majority of studies defined complicated UTI based on both complicating host factors and systemic involvement, different clinical phenotypes were included in each study. This not only contributes further to disparities between studies, it also affects the applicability of study results. Moreover, the aforementioned heterogeneity is compounded by the fact that host factors are very diverse in themselves and there is no consensus about which host factors should be included in the definition of complicated UTI. As astutely phrased by James Johnson [67], "it may be time to find a different term than complicated UTI for UTIs that occur in patients with underlying predisposing factors, since this term seems hopelessly mired in ambiguity." Johansen et al. [68] have proposed a UTI classification system for clinical and research purposes based on clinical phenotype, severity, host factors and pathogen susceptibility. However, this classification system was not used by any of the included studies in our review. In the Netherlands, the primary care guidelines for UTI have already made a distinction between a UTI in a complicated host versus UTI with systemic involvement. [69]

Existing research guidelines

We found that few studies met symptom, pyuria and urine culture criteria mentioned in FDA and EMA guidelines. [3-5] In addition, we identified that studies more frequently based UTI definitions on clinical practice guidelines. The use of clinical practice guidelines in the place of research guidelines seems inappropriate, as clinical guidelines are less stringent than research guidelines, and they base empirical treatment recommendations on limited diagnostic information. Taken together, our findings imply that a widely accepted, consensus-based gold

standard for the diagnosis of UTI is lacking, and is much needed in the field of UTI research.

Strengths and limitations

Strengths of this systematic review include our comprehensive search strategy, including multiple electronic databases, and extracting data from supplemental material, as UTI definitions were frequently only mentioned in a supplemental protocol. Our study has several limitations. For some of the included therapeutic studies, eligibility criteria served as a proxy for the UTI definition, if a definition was not mentioned separately. This might have contributed to additional heterogeneity. For instance, prophylactic studies including patients with recurrent UTI more frequently provided separate UTI definitions, since these often served as outcome measures. Also, some heterogeneity might be explained by the fact that we included studies that investigated different UTI phenotypes. However, this effect was mitigated by evaluating different UTI phenotypes separately. Another limitation is that we filtered our search strategy on publication date and study type. While expanding the time period would have provided more data, it would not reflect the most recent consensus and would likely have contributed to further heterogeneity, as these studies were published before the FDA and EMA guidance documents. Furthermore, including more observational studies most likely would not have reduced heterogeneity, as these are presumably less likely to follow FDA and EMA guidelines for drug approval. Since we did not find any recent studies that were conducted in long-term care facilities, and we excluded studies regarding CA-UTI and UTI in spinal cord injury patients, it is unclear how heterogeneous definitions are in these areas. Defining UTI might be even more challenging for these populations and settings.

Conclusions

In conclusion, UTI definitions differ widely across recent therapeutic and interventional studies. An international consensus-based reference standard is needed to reduce misclassification bias within studies and heterogeneity between studies. To avoid ambiguity, such a reference standard should veer away from the term ‘complicated UTI’ and instead categorise UTI based on systemic involvement, as these are different entities with different treatments. Based on results of this systematic review, our group has initiated an international consensus study to construct a UTI reference standard for research purposes.

Funding

No funding was received for this project.

Author contributions

Conceptualisation and methodology M.P.B., R.M.H.J., S.P.C., L.G.V., M.M.C.L.; screening and data extraction M.P.B., R.M.H.J.; data analysis M.P.B.; writing – original draft preparation M.P.B., R.M.H.J; writing – review and editing M.P.B, R.M.H.J., C.S, T.N.P., C.N, L.M, J.M.C, S.E.G, B.K., F.W., S.P.C, L.G.V., M.M.C.L.; supervision M.M.C.L., S.P.C, and L.G.V. All authors have read and agreed to the final version of the manuscript.

Acknowledgements

The authors would like to thank J.W. Schoones for his contribution to the search strategy.

Conflicts of interest

None of the authors have an association that might pose a conflict of interest regarding this manuscript.

References

1. Gupta K, Grigoryan L, Trautner B. Urinary Tract Infection. Ann Intern Med **2017**; 167(7): ITC49-ITC64.
2. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am **2014**; 28(1): 1-13.
3. Evaluation of medicinal products indicated for treatment of bacterial infections. Available at: https://www.ema.europa.eu/en/evaluation-medicinal-products-indicated-treatment-bacterial-infections Accessed 8th of June.
4. Uncomplicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/uncomplicated-urinary-tract-infections-developing-drugs-treatment-guidance-industry Accessed 8th of June.
5. Complicated Urinary Tract Infections: Developing Drugs for Treatment. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/complicated-urinary-tract-infections-developing-drugs-treatment Accessed 8th of June.
6. Stone ND, Ashraf MS, Calder J, et al. Surveillance definitions of infections in long-term care facilities: revisiting the McGeer criteria. Infect Control Hosp Epidemiol **2012**; 33(10): 965-77.
7. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis **2011**; 52(5): e103-20.
8. Bonkat G, Bartoletti F, Bruyere F, et al. EAU Guidelines on Urological Infections. Available at: https://uroweb.org/guidelines/urological-infections. Accessed 25th of February 2023.
9. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ **2021**; 372: n71.
10. Aloush SM, Al-Awamreh K, Abu Sumaqa Y, Halabi M, Al Bashtawy M, Salama FB. Effectiveness of antibiotics versus ibuprofen in relieving symptoms of nosocomial urinary tract infection: A comparative study. J Am Assoc Nurse Pract **2019**; 31(1): 60-4.
11. Arakawa S, Kawahara K, Kawahara M, et al. The efficacy and safety of tazobactam/ceftolozane in Japanese patients with uncomplicated pyelonephritis and complicated urinary tract infection. J Infect Chemother **2019**; 25(2): 104-10.
12. Babar A, Moore L, Leblanc V, et al. High dose versus low dose standardized cranberry proanthocyanidin extract for the prevention of recurrent urinary tract infection in healthy women: a double-blind randomized controlled trial. BMC Urol **2021**; 21(1): 44.
13. Boel JB, Antsupova V, Knudsen JD, Jarløv JO, Arpi M, Holzknecht BJ. Intravenous mecillinam compared with other β-lactams as targeted treatment for Escherichia coli or Klebsiella spp. bacteraemia with urinary tract focus. J Antimicrob Chemother **2021**; 76(1): 206-11.
14. Botros C, Lozo S, Iyer S, et al. Methenamine hippurate compared with trimethoprim for the prevention of recurrent urinary tract infections: a randomized clinical trial. Int Urogynecol J **2022**; 33(3): 571-80.

15. Bruyère F, Azzouzi AR, Lavigne JP, et al. A Multicenter, Randomized, Placebo-Controlled Study Evaluating the Efficacy of a Combination of Propolis and Cranberry (Vaccinium macrocarpon) (DUAB®) in Preventing Low Urinary Tract Infection Recurrence in Women Complaining of Recurrent Cystitis. Urol Int **2019**; 103(1): 41-8.

16. Costache RC, Novac B, Bardan TR, Agapie DN, Edu A. Xyloglucan + Gelose Combination versus Placebo as Adjuvant Therapy to First-Line Antimicrobials for Uncomplicated Urinary Tract Infection in Adults. Urol Int **2019**; 102(4): 468-75.

17. Diebold R, Schopf B, Stammer H, Mendling W. Vaginal treatment with lactic acid gel delays relapses in recurrent urinary tract infections: results from an open, multicentre observational study. Archives of Gynecology and Obstetrics **2021**; 304(2): 409-17.

18. Drekonja DM, Trautner B, Amundson C, Kuskowski M, Johnson JR. Effect of 7 vs 14 Days of Antibiotic Therapy on Resolution of Symptoms Among Afebrile Men With Urinary Tract Infection: A Randomized Clinical Trial. Jama **2021**; 326(4): 324-31.

19. Eckburg PB, Muir L, Critchley IA, et al. Oral Tebipenem Pivoxil Hydrobromide in Complicated Urinary Tract Infection. N Engl J Med **2022**; 386(14): 1327-38.

20. Edlund C, Ternhag A, Skoog Ståhlgren G, et al. The clinical and microbiological efficacy of temocillin versus cefotaxime in adults with febrile urinary tract infection, and its effects on the intestinal microbiota: a randomised multicentre clinical trial in Sweden. Lancet Infect Dis **2022**; 22(3): 390-400.

21. El Nekidy WS, Abdelsalam MM, Nusair AR, et al. Is Cefoxitin a Carbapenem Sparing Agent in the Management of Urinary Tract Infections Caused by ESBL Producing Enterobacterales? Hospital Pharmacy **2021**.

22. Ferrante KL, Wasenda EJ, Jung CE, Adams-Piper ER, Lukacz ES. Vaginal Estrogen for the Prevention of Recurrent Urinary Tract Infection in Postmenopausal Women: A Randomized Clinical Trial. Female Pelvic Med Reconstr Surg **2021**; 27(2): 112-7.

23. Gama CRB, Pombo MAG, Nunes CP, et al. Treatment of Recurrent Urinary Tract Infection Symptoms with Urinary Antiseptics Containing Methenamine and Methylene Blue: Analysis of Etiology and Treatment Outcomes. Res Rep Urol **2020**; 12: 639-49.

24. Gamble KC, Rose DT, Sapozhnikov J. Intravenous to oral antibiotics versus intravenous antibiotics: a step-up or a step-down for extended spectrum beta-lactamase (ESBL)-producing urinary tract infections without concomitant bacteraemia? International Journal of Antimicrobial Agents **2022**; 59(3) (no pagination).

25. Gágyor I, Hummers E, Schmiemann G, et al. Herbal treatment with uva ursi extract versus fosfomycin in women with uncomplicated urinary tract infection in primary care: a randomized controlled trial. Clin Microbiol Infect **2021**; 27(10): 1441-7.

26. Harding C, Chadwick T, Homer T, et al. Methenamine hippurate compared with antibiotic prophylaxis to prevent recurrent urinary tract infections in women: the ALTAR non-inferiority RCT. Health Technol Assess **2022**; 26(23): 1-172.

27. Jansaker F, Thonnings S, Hertz FB, et al. Three versus five days of pivmecillinam for community-acquired uncomplicated lower urinary tract infection: A randomised, double-blind, placebo-controlled superiority trial. EClinicalMedicine **2019**; 12: 62-9.

28. Kaye KS, Rice LB, Dane AL, et al. Fosfomycin for Injection (ZTI-01) Versus Piperacillin-tazobactam for the Treatment of Complicated Urinary Tract Infection Including Acute Pyelonephritis: ZEUS, A Phase 2/3 Randomized Trial. Clin Infect Dis **2019**; 69(12): 2045-56.

29. Kohno S, Bando H, Yoneyama F, et al. The safety and efficacy of relebactam/imipenem/cilastatin in Japanese patients with complicated intra-abdominal infection or complicated urinary tract infection: A multicenter, open-label, noncomparative phase 3 study. J Infect Chemother **2021**; 27(2): 262-70.

30. Koradia P, Kapadia S, Trivedi Y, Chanchu G, Harper A. Probiotic and cranberry supplementation for preventing recurrent uncomplicated urinary tract infections in premenopausal women: a controlled pilot study. Expert Rev Anti Infect Ther **2019**; 17(9): 733-40.

31. Li Y, Yin Y, Peng X, et al. A randomized, active-controlled, multicentre clinical trial to evaluate the efficacy and safety of oral sitafloxacin versus levofloxacin in Chinese adults with acute uncomplicated or complicated urinary tract infection. Ann Med **2021**; 53(1): 217-26.

32. Lojanapiwat B, Nimitvilai S, Bamroongya M, et al. Oral sitafloxacin vs intravenous ceftriaxone followed by oral cefdinir for acute pyelonephritis and complicated urinary tract infection: a randomized controlled trial. Infect Drug Resist **2019**; 12: 173-81.

33. Mir MA, Chaudhary S, Payasi A, Sood R, Mavuduru RS, Shameem M. Ceftriaxone+Sulbactam+Disodium EDTA Versus Meropenem for the Treatment of Complicated Urinary Tract Infections, Including Acute Pyelonephritis: PLEA, a Double-Blind, Randomized Noninferiority Trial. Open Forum Infectious Diseases **2019**; 6(10) (no pagination).

34. Mirzaei M, Daneshpajooh A, Farsinezhad A, et al. The Therapeutic Effect of Intravesical Instillation of Platelet Rich Plasma on Recurrent Bacterial Cystitis in Women: A Randomized Clinical Trial. Urol J **2019**; 16(6): 609-13.

35. Montelin H, Forsman KJ, Tängdén T. Retrospective evaluation of nitrofurantoin and pivmecillinam for the treatment of lower urinary tract infections in men. PLoS One **2019**; 14(1): e0211098.

36. Nestler S, Grune B, Schilchegger L, Suna A, Perez A, Neisius A. Efficacy of vaccination with StroVac for recurrent urinary tract infections in women: a comparative single-centre study. International Urology and Nephrology **2021**; 53(11): 2267-72.

37. Overcash JS, Tiffany CA, Scangarella-Oman NE, et al. Phase 2a Pharmacokinetic, Safety, and Exploratory Efficacy Evaluation of Oral Gepotidacin (GSK2140944) in Female Participants with Uncomplicated Urinary Tract Infection (Acute Uncomplicated Cystitis). Antimicrob Agents Chemother **2020**; 64(7).

38. Overcash JS, Bhiwandi P, Garrity-Ryan L, et al. Pharmacokinetics, Safety, and Clinical Outcomes of Omadacycline in Women with Cystitis: Results from a Phase 1b Study. Antimicrob Agents Chemother **2019**; 63(5).

39. Pierrotti LC, Perez-Nadales E, Fernandez-Ruiz M, et al. Efficacy of beta-lactam/beta-lactamase inhibitors to treat extended-spectrum beta-lactamase-producing Enterobacterales bacteremia secondary to urinary tract infection in kidney transplant recipients (INCREMENT-SOT Project). Transplant Infectious Disease **2020**.

40. Rădulescu D, David C, Turcu FL, Spătaru DM, Popescu P, Văcăroiu IA. Combination of cranberry extract and D-mannose - possible enhancer of uropathogen sensitivity to antibiotics in acute therapy of urinary tract infections: Results of a pilot study. Exp Ther Med **2020**; 20(4): 3399-406.

41. Ryanto S, Wong M, Czarniak P, et al. The use of initial dosing of gentamicin in the management of pyelonephritis/urosepsis: A retrospective study. PLoS One **2019**; 14(1): e0211094.

42. Safwat AS, Hasanain A, Shahat A, et al. Cholecalciferol for the prophylaxis against recurrent urinary tract infection among patients with benign prostatic hyperplasia: a randomized, comparative study. World J Urol **2019**; 37(7): 1347-52.

43. Sagan O, Yakubsevitch R, Yanev K, et al. Pharmacokinetics and Tolerability of Intravenous Sulbactam-Durlobactam with Imipenem-Cilastatin in Hospitalized Adults with Complicated Urinary Tract Infections, Including Acute Pyelonephritis. Antimicrob Agents Chemother **2020**; 64(3).

44. Sashidhar RB, Manoj KYM, Manisha S, Sree SRS, Kamala SH. Assessment of Efficacy and Safety of Oral Fosfomycin Single Dose in Uncomplicated Urinary Tract Infection at a Tertiary Care Hospital in South India. Asian Journal of Pharmaceutical and Clinical Research **2022**; 15(2): 60-3.

45. Senard O, Lafaurie M, Lesprit P, et al. Efficacy of cefoxitin versus carbapenem in febrile male urinary tract infections caused by extended spectrum beta-lactamase-producing Escherichia coli: a multicenter retrospective cohort study with propensity score analysis. European Journal of Clinical Microbiology and Infectious Diseases **2020**; 39(1): 121-9.

46. Sharara SL, Amoah J, Pana ZD, Simner PJ, Cosgrove SE, Tamma PD. Is Piperacillin-Tazobactam Effective for the Treatment of Pyelonephritis Caused by Extended-Spectrum beta-Lactamase-Producing Organisms? Clinical Infectious Diseases **2020**; 71(8): E331-E7.

47. Sojo-Dorado J, López-Hernández I, Rosso-Fernandez C, et al. Effectiveness of Fosfomycin for the Treatment of Multidrug-Resistant Escherichia coli Bacteremic Urinary Tract Infections: A Randomized Clinical Trial. JAMA Netw Open **2022**; 5(1): e2137277.

48. Sorli L, Luque S, Li J, et al. Colistin for the treatment of urinary tract infections caused by extremely drug-resistant Pseudomonas aeruginosa: Dose is critical. Journal of Infection **2019**; 79(3): 253-61.

49. Stalenhoef JE, van Nieuwkoop C, Menken PH, Bernards ST, Elzevier HW, van Dissel JT. Intravesical Gentamicin Treatment for Recurrent Urinary Tract Infections Caused by Multidrug Resistant Bacteria. J Urol **2019**; 201(3): 549-55.

50. Tehrani S, Elyasi F, Abolghasemi S. Levofloxacin Versus Ceftriaxone for the Treatment of Acute Pyelonephritis in Iranian Adults. Infect Disord Drug Targets **2021**; 21(4): 603-7.

51. Ten Doesschate T, van Werkhoven H, Meijvis S, et al. Fosfomycin-trometamol for Urinary Tract Infections in Kidney Transplant Recipients. Transplantation **2019**; 103(6): 1272-6.

52. Ten Doesschate T, Kuiper S, van Nieuwkoop C, et al. Fosfomycin versus ciprofloxacin as oral stepdown treatment for Escherichia coli febrile urinary tract infection in women: a randomised, placebo-controlled, double blind, multicenter trial. Clin Infect Dis **2021**.

53. Tseng CS, Chang SJ, Meng E, Chang HC, Lee YJ. The efficacy of pentosan polysulfate monotherapy for preventing recurrent urinary tract infections in women: A multicenter open-label randomized controlled trial. J Formos Med Assoc **2020**; 119(8): 1314-9.

54. Tullos JB, Stoudenmire LL, Pouliot JD. Piperacillin-Tazobactam Versus Carbapenems for the Treatment of Nonbacteremic Urinary Tract Infections due to Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae. Hospital Pharmacy **2020**; 55(1): 44-9.

55. Wagenlehner FME, Cloutier DJ, Komirenko AS, et al. Once-Daily Plazomicin for Complicated Urinary Tract Infections. N Engl J Med **2019**; 380(8): 729-40.

56. Wald-Dickler N, Lee TC, Tangpraphaphorn S, et al. Fosfomycin vs Ertapenem for Outpatient Treatment of Complicated Urinary Tract Infections: A Multicenter, Retrospective Cohort Study. Open Forum Infect Dis **2022**; 9(1): ofab620.

57. Nicolle LE, Bradley S, Colgan R, et al. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis **2005**; 40(5): 643-54.

58. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis **2008**; 40(10): 804-10.

59. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc **1995**; 43(7): 772-5.

60. Bent S, Nallamothu BK, Simel DL, Fihn SD, Saint S. Does this woman have an acute uncomplicated urinary tract infection? JAMA **2002**; 287(20): 2701-10.

61. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med **2013**; 369(20): 1883-91.

62. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med **1982**; 307(8): 463-8.

63. Klaassen IL, de Haas V, van Wijk JA, Kaspers GJ, Bijlsma M, Bokenkamp A. Pyuria is absent during urinary tract infections in neutropenic patients. Pediatr Blood Cancer **2011**; 56(5): 868-70.

64. Bilsen MP, Aantjes MJ, van Andel E, et al. Current pyuria cut-offs promote inappropriate UTI diagnosis in older women. Clin Infect Dis **2023**.

65. van den Broek D, Keularts IM, Wielders JP, Kraaijenhagen RJ. Benefits of the iQ200 automated urine microscopy analyser in routine urinalysis. Clin Chem Lab Med **2008**; 46(11): 1635-40.

66. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis **2004**; 38(8): 1150-8.

67. Johnson JR. Definition of Complicated Urinary Tract Infection. Clin Infect Dis **2017**; 64(4): 529.

68. Johansen TE, Botto H, Cek M, et al. Critical review of current definitions of urinary tract infections and proposal of an EAU/ESIU classification system. Int J Antimicrob Agents **2011**; 38 Suppl: 64-70.

69. Nederlands Huisartsen Genootschap (NHG) standaard: urineweginfecties (Dutch primary care guideline: UTI). Available at: https://richtlijnen.nhg.org/standaarden/urineweginfecties. Accessed 15th of March 2023.

Supplement

Search strategy

Search date: May 16th, 2022

Number of articles per electronic database:

- **PubMed**: 308 – after removal of comments, editorials and letter: 294 – after applying publication year filter (2019 and more recent): 190
- **Embase**: 327 – 161 unique articles – after applying publication year filter: 119
- **Web of Science**: 165 – 17 unique articles – after applying publication year filter: 15
- **Cochrane library** (published trials only): 241 – 40 unique – after applying publication year filter: 24

Search strategy per electronic database:

PubMed

http://www.ncbi.nlm.nih.gov/pubmed?otool=leiden

((("Anti-Bacterial Agents"[Mesh] OR "Anti-Bacterial Agents"[Pharmacological Action] OR "Anti-Bacterial Agents"[tw] OR "Anti-Bacterial Agent"[tw] OR "Antibacterial Agents"[tw] OR "Antibacterial Agent"[tw] OR "antibiotic"[tw] OR "antibiotics"[tw] OR "anti biotic"[tw] OR "anti biotics"[tw] OR "Fluoroquinolones"[Mesh] OR "Fluoroquinolones"[tw] OR "Fluoroquinolone"[tw] OR "Ciprofloxacin"[tw] OR "Enoxacin"[tw] OR "Enrofloxacin"[tw] OR "Fleroxacin"[tw] OR "Gatifloxacin"[tw] OR "Gemifloxacin"[tw] OR "Levofloxacin"[tw] OR "Moxifloxacin"[tw] OR "Norfloxacin"[tw] OR "Ofloxacin"[tw] OR "Pefloxacin"[tw] OR "Fosfomycin"[Mesh] OR "Fosfomycin"[tw] OR "Phosphomycin"[tw] OR "Phosphonomycin"[tw] OR "Monuril"[tw] OR "Cephalosporins"[Mesh] OR "Cephalosporins"[tw] OR "Cephalosporin"[tw] OR "Cefaclor"[tw] OR "Cefadroxil"[tw] OR "Cefamandole"[tw] OR "Cefatrizine"[tw] OR "Cefazolin"[tw] OR "Cefdinir"[tw] OR "Cefepime"[tw] OR "Cefixime"[tw] OR "Cefmenoxime"[tw] OR "Cefmetazole"[tw] OR "Cefonicid"[tw] OR "Cefoperazone"[tw] OR "Cefotaxime"[tw] OR "Cefotetan"[tw] OR "Cefotiam"[tw] OR "Cefoxitin"[tw] OR "Cefsulodin"[tw] OR "Ceftazidime"[tw] OR "Ceftibuten"[tw] OR "Ceftizoxime"[tw] OR "Ceftriaxone"[tw] OR "Cefuroxime"[tw] OR "Cephacetrile"[tw] OR "Cephalexin"[tw] OR "Cephaloglycin"[tw] OR "Cephaloridine"[tw] OR "Cephalothin"[tw] OR "Cephamycins"[tw] OR "Cephapirin"[tw] OR "Cephradine"[tw] OR "Carbapenems"[Mesh] OR "Carbapenems"[tw] OR "Carbapenem"[tw] OR "Doripenem"[tw] OR "Ertapenem"[tw] OR "Thienamycins"[tw] OR "Imipenem"[tw] OR "Meropenem"[tw] OR "Aminoglycosides"[mesh:noexp] OR "Gentamicins"[mesh] OR "Tobramycin"[Mesh] OR "Aminoglycosides"[tw] OR "aminoglycoside"[tw] OR "gentamycin"[tw] OR "Gentamycins"[tw] OR "Gentamicin"[tw] OR "Gentamicins"[tw] OR "Sisomicin"[tw] OR "Netilmicin"[tw] OR "tobramycin"[tw] OR "Tobramycins"[tw] OR "Vaccinium macrocarpon"[Mesh] OR "Vaccinium macrocarpon"[tw] OR "cranberry"[tw] OR "cranberries"[tw] OR "cranberr*"[tw] OR "Methenamine"[Mesh] OR "Methenamine"[tw] OR "Hexamine"[tw] OR "Hexamethylenetetramine"[tw] OR "Urotropin"[tw] OR "Aminoform"[tw]

OR "Mannose"[Mesh] OR "Mannose"[tw] OR "D-Mannose"[tw] OR "Mannopyranoside"[tw] OR "Mannopyranose"[tw] OR "vaginal oestrogen"[tw] OR "vaginal oestrogens"[tw] OR "vaginal estrogen"[tw] OR "vaginal estrogens"[tw] OR ((("Administration, Intravaginal"[Mesh] OR "Vaginal Creams, Foams, and Jellies"[mesh] OR "Vaginal Absorption"[mesh] OR "vaginal"[tw] OR "vagina"[tw] OR "intravaginal"[tw]) AND ("Estrogens"[mesh] OR "Estrogens"[pharmacological action] OR "oestrogen"[tw] OR "oestrogens"[tw] OR "estrogen"[tw] OR "estrogens"[tw])) OR "drug therapy"[subheading] OR "drug therapy"[mesh]) AND ("Urinary Tract Infections"[majr:noexp] OR "Urinary Tract Infection"[ti] OR "Urinary Tract Infections"[ti] OR "Urinary Infection"[ti] OR "Urinary Infections"[ti] OR "Urogenital Tract Infection"[ti] OR "Urogenital Tract Infections"[ti] OR "Urogenital Infection"[ti] OR "Urogenital Infections"[ti] OR "Pyuria"[majr] OR "Pyuria"[ti] OR "Pyurias"[ti] OR (("Cystitis"[majr:noexp] OR "Cystitis"[ti] OR "Pyelocystitis"[majr] OR "Pyelocystitis"[ti]) NOT ("Cystitis, Interstitial"[majr] OR "Interstitial Cystitis"[ti])) OR (("Pyelonephritis"[majr] OR "pyelonephritis"[ti]) NOT ("Pyelonephritis, Xanthogranulomatous"[majr] OR "Xanthogranulomatous Pyelonephritis"[ti])) OR "urosepsis"[ti] OR "urinary sepsis"[ti] OR "urinary tract sepsis"[ti] OR "uroseptic"[ti]) NOT (("Infant"[mesh] OR "Child"[mesh] OR "Adolescent"[mesh] OR "Infant"[ti] OR "Infants"[ti] OR "Child"[ti] OR "Children"[ti] OR "Adolescent"[ti] OR "Adolescents"[ti] OR "pediatric"[ti] OR "paediatric"[ti] OR "pediatric*"[ti] OR "paediatric*"[ti]) NOT ("Adult"[mesh] OR "adult"[ti] OR "adults"[ti] OR "elderly"[ti])) AND ("2017/01/01"[PDAT] : "3000/12/31"[PDAT]) AND (randomized controlled trial[pt] OR controlled clinical trial[pt] OR clinical trial[pt] OR randomized[tiab] OR randomised[tiab] OR placebo[tiab] OR clinical trials as topic[mesh:noexp] OR randomly[tiab] OR trial[ti] OR "RCT"[ti]) NOT (animals[mh] NOT humans [mh]) NOT ((systematic[sb] OR "review"[pt]) NOT clinical trial[pt]))

Broader search:

((("Anti-Bacterial Agents"[Mesh] OR "Anti-Bacterial Agents"[Pharmacological Action] OR "Anti-Bacterial Agents"[tw] OR "Anti-Bacterial Agent"[tw] OR "Antibacterial Agents"[tw] OR "Antibacterial Agent"[tw] OR "antibiotic"[tw] OR "antibiotics"[tw] OR "anti biotic"[tw] OR "anti biotics"[tw] OR "Fluoroquinolones"[Mesh] OR "Fluoroquinolones"[tw] OR "Fluoroquinolone"[tw] OR "Ciprofloxacin"[tw] OR "Enoxacin"[tw] OR "Enrofloxacin"[tw] OR "Fleroxacin"[tw] OR "Gatifloxacin"[tw] OR "Gemifloxacin"[tw] OR "Levofloxacin"[tw] OR "Moxifloxacin"[tw] OR "Norfloxacin"[tw] OR "Ofloxacin"[tw] OR "Pefloxacin"[tw] OR "Fosfomycin"[Mesh] OR "Fosfomycin"[tw] OR "Phosphomycin"[tw] OR "Phosphonomycin"[tw] OR "Monuril"[tw] OR "Cephalosporins"[Mesh] OR "Cephalosporins"[tw] OR "Cephalosporin"[tw] OR "Cefaclor"[tw] OR "Cefadroxil"[tw] OR "Cefamandole"[tw] OR "Cefatrizine"[tw] OR "Cefazolin"[tw] OR "Cefdinir"[tw] OR "Cefepime"[tw] OR "Cefixime"[tw] OR "Cefmenoxime"[tw] OR "Cefmetazole"[tw] OR "Cefonicid"[tw] OR "Cefoperazone"[tw] OR "Cefotaxime"[tw] OR "Cefotetan"[tw] OR "Cefotiam"[tw] OR "Cefoxitin"[tw] OR "Cefsulodin"[tw] OR "Ceftazidime"[tw] OR "Ceftibuten"[tw] OR "Ceftizoxime"[tw] OR "Ceftriaxone"[tw] OR "Cefuroxime"[tw] OR "Cephacetrile"[tw] OR "Cephalexin"[tw] OR "Cephaloglycin"[tw] OR "Cephaloridine"[tw] OR "Cephalothin"[tw] OR "Cephamycins"[tw] OR "Cephapirin"[tw] OR "Cephradine"[tw] OR "Carbapenems"[Mesh] OR "Carbapenems"[tw] OR "Carbapenem"[tw] OR "Doripenem"[tw] OR "Ertapenem"[tw] OR "Thienamycins"[tw] OR "Imipenem"[tw] OR "Meropenem"[tw] OR "Vaccinium macrocarpon"[Mesh] OR "Vaccinium macrocarpon"[tw] OR "cranberry"[tw] OR "cranberries"[tw] OR "cranberr*"[tw] OR "Methenamine"[Mesh] OR "Methenamine"[tw] OR "Hexamine"[tw] OR "Hexamethylenetetramine"[tw] OR "Urotropin"[tw] OR "Aminoform"[tw]

OR "Mannose"[Mesh] OR "Mannose"[tw] OR "D-Mannose"[tw] OR "Mannopyranoside"[tw] OR "Mannopyranose"[tw] OR "vaginal oestrogen"[tw] OR "vaginal oestrogens"[tw] OR "vaginal estrogen"[tw] OR "vaginal estrogens"[tw] OR (("Administration, Intravaginal"[Mesh] OR "Vaginal Creams, Foams, and Jellies"[mesh] OR "Vaginal Absorption"[mesh] OR "vaginal"[tw] OR "vagina"[tw] OR "intravaginal"[tw]) AND ("Estrogens"[mesh] OR "Estrogens"[pharmacological action] OR "oestrogen"[tw] OR "oestrogens"[tw] OR "estrogen"[tw] OR "estrogens"[tw])) OR "drug therapy"[subheading] OR "drug therapy"[mesh]) AND ("Urinary Tract Infections"[Mesh:noexp] OR "Urinary Tract Infection"[tw] OR "Urinary Tract Infections"[tw] OR "Urinary Infection"[tw] OR "Urinary Infections"[tw] OR "Urogenital Tract Infection"[tw] OR "Urogenital Tract Infections"[tw] OR "Urogenital Infection"[tw] OR "Urogenital Infections"[tw] OR "Pyuria"[mesh] OR "Pyuria"[tw] OR "Pyurias"[tw] OR (("Cystitis"[mesh:noexp] OR "Cystitis"[tw] OR "Pyelocystitis"[mesh] OR "Pyelocystitis"[tw]) NOT ("Cystitis, Interstitial"[majr] OR "Interstitial Cystitis"[ti])) OR (("Pyelonephritis"[Mesh] OR "pyelonephritis"[tw]) NOT ("Pyelonephritis, Xanthogranulomatous"[majr] OR "Xanthogranulomatous Pyelonephritis"[ti])) OR "urosepsis"[tw] OR "urinary sepsis"[tw] OR "urinary tract sepsis"[tw] OR "uroseptic"[tw]) NOT (("Infant"[mesh] OR "Child"[mesh] OR "Adolescent"[mesh] OR "Infant"[ti] OR "Infants"[ti] OR "Child"[ti] OR "Children"[ti] OR "Adolescent"[ti] OR "Adolescents"[ti] OR "pediatric"[ti] OR "paediatric"[ti] OR "pediatric*"[ti] OR "paediatric*"[ti]) NOT ("Adult"[mesh] OR "adult"[ti] OR "adults"[ti] OR "elderly"[ti])) AND ("2017/01/01"[PDAT] : "3000/12/31"[PDAT]) AND (randomized controlled trial[pt] OR controlled clinical trial[pt] OR clinical trial[pt] OR randomized[tiab] OR randomised[tiab] OR placebo[tiab] OR clinical trials as topic[mesh:noexp] OR randomly[tiab] OR trial[ti]) NOT (animals[mh] NOT humans [mh]) NOT (systematic[sb] NOT clinical trial[pt]))

Embase

http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=main&MODE=ovid&D=oemezd

((exp *"Antibiotic agent"/ OR "Anti-Bacterial Agents".ti,ab OR "Anti-Bacterial Agent".ti,ab OR "Antibacterial Agents".ti,ab OR "Antibacterial Agent".ti,ab OR "antibiotic".ti,ab OR "antibiotics".ti,ab OR "anti biotic".ti,ab OR "anti biotics".ti,ab OR exp *"quinolone derivative"/ OR "Fluoroquinolones".ti,ab OR "Fluoroquinolone".ti,ab OR "Ciprofloxacin".ti,ab OR "Enoxacin".ti,ab OR "Enrofloxacin".ti,ab OR "Fleroxacin".ti,ab OR "Gatifloxacin".ti,ab OR "Gemifloxacin".ti,ab OR "Levofloxacin".ti,ab OR "Moxifloxacin".ti,ab OR "Norfloxacin".ti,ab OR "Ofloxacin".ti,ab OR "Pefloxacin".ti,ab OR exp *"Fosfomycin"/ OR "Fosfomycin".ti,ab OR "Phosphomycin".ti,ab OR "Phosphonomycin".ti,ab OR "Monuril".ti,ab OR exp *"Cephalosporin Derivative"/ OR "Cephalosporins".ti,ab OR "Cephalosporin".ti,ab OR "Cefaclor".ti,ab OR "Cefadroxil".ti,ab OR "Cefamandole".ti,ab OR "Cefatrizine".ti,ab OR "Cefazolin".ti,ab OR "Cefdinir".ti,ab OR "Cefepime".ti,ab OR "Cefixime".ti,ab OR "Cefmenoxime".ti,ab OR "Cefmetazole".ti,ab OR "Cefonicid".ti,ab OR "Cefoperazone".ti,ab OR "Cefotaxime".ti,ab OR "Cefotetan".ti,ab OR "Cefotiam".ti,ab OR "Cefoxitin".ti,ab OR "Cefsulodin".ti,ab OR "Ceftazidime".ti,ab OR "Ceftibuten".ti,ab OR "Ceftizoxime".ti,ab OR "Ceftriaxone".ti,ab OR "Cefuroxime".ti,ab OR "Cephacetrile".ti,ab OR "Cephalexin".ti,ab OR "Cephaloglycin".ti,ab OR "Cephaloridine".ti,ab OR "Cephalothin".ti,ab OR "Cephamycins".ti,ab OR "Cephapirin".ti,ab OR "Cephradine".ti,ab OR "Carbapenems"/ OR "Carbapenems".ti,ab OR "Carbapenem".ti,ab OR "Doripenem".ti,ab OR "Ertapenem".ti,ab OR "Thienamycins".ti,ab OR "Imipenem".ti,ab OR "Meropenem".ti,ab OR exp *"Aminoglycoside"/ OR exp *"Aminoglycoside Antibiotic agent"/ OR exp *"Gentamicin"/ OR exp *"Tobramycin"/ OR "Aminoglycosides".ti,ab OR "aminoglycoside".ti,ab OR "gentamycin".ti,ab OR "Gentamycins".ti,ab OR "Gentamicin".ti,ab OR "Gentamicins".ti,ab OR "Sisomicin".ti,ab OR

"Netilmicin".ti,ab OR "tobramycin".ti,ab OR "Tobramycins".ti,ab OR exp *"cranberry extract"/ OR *"cranberry"/ OR *"cranberry juice"/ OR "Vaccinium macrocarpon".ti,ab OR "cranberry".ti,ab OR "cranberries".ti,ab OR "cranberr*".ti,ab OR exp *"Methenamine"/ OR "Methenamine".ti,ab OR "Hexamine".ti,ab OR "Hexamethylenetetramine".ti,ab OR "Urotropin".ti,ab OR "Aminoform".ti,ab OR exp *"Mannose"/ OR "Mannose".ti,ab OR "D-Mannose".ti,ab OR "Mannopyranoside".ti,ab OR "Mannopyranose".ti,ab OR "vaginal oestrogen".ti,ab OR "vaginal oestrogens".ti,ab OR "vaginal estrogen".ti,ab OR "vaginal estrogens".ti,ab OR ((exp *"intravaginal drug administration"/ OR exp *"agents used intravaginally"/ OR "vaginal".ti,ab OR "vagina".ti,ab OR "intravaginal".ti,ab) AND (exp "Estrogen"/ OR "oestrogen".ti,ab OR "oestrogens".ti,ab OR "estrogen".ti,ab OR "estrogens".ti,ab)) OR exp *"drug therapy"/) AND (*"Urinary Tract Infection"/ OR "Urinary Tract Infection".ti OR "Urinary Tract Infections".ti OR "Urinary Infection".ti OR "Urinary Infections".ti OR "Urogenital Tract Infection".ti OR "Urogenital Tract Infections".ti OR "Urogenital Infection".ti OR "Urogenital Infections".ti OR *"Pyuria"/ OR "Pyuria".ti OR "Pyurias".ti OR ((*"Cystitis"/ OR "Cystitis".ti OR "Pyelocystitis"/ OR "Pyelocystitis".ti) NOT (*"Interstitial Cystitis"/ OR "Interstitial Cystitis".ti)) OR ((exp *"Pyelonephritis"/ OR "pyelonephritis".ti) NOT (*"Xanthogranulomatous Pyelonephritis"/ OR "Xanthogranulomatous Pyelonephritis".ti)) OR exp *"urosepsis"/ OR "urosepsis".ti OR "urinary sepsis".ti OR "urinary tract sepsis".ti OR "uroseptic".ti) NOT ((exp "Infant"/ OR exp "Child"/ OR exp "Adolescent"/ OR "Infant".ti OR "Infants".ti OR "Child".ti OR "Children".ti OR "Adolescent".ti OR "Adolescents".ti OR "pediatric".ti OR "paediatric".ti OR "pediatric*".ti OR "paediatric*".ti) NOT (exp "Adult"/ OR "adult".ti OR "adults".ti OR "elderly".ti)) AND 2017:2023.(sa_year) AND (exp "randomized controlled trial"/ OR exp "controlled clinical trial"/ OR exp "clinical trial"/ OR randomized.ti OR randomised.ti OR placebo.ti OR randomly.ti OR trial.ti OR "RCT".ti) NOT (exp "animals"/ NOT exp "humans"/) NOT ((exp "systematic review"/ OR exp "review"/) NOT exp "clinical trial"/) NOT (conference review or conference abstract).pt)

Web of Science

http://isiknowledge.com/wos ((TI=("Antibiotic agent" OR "Anti-Bacterial Agents" OR "Anti-Bacterial Agent" OR "Antibacterial Agents" OR "Antibacterial Agent" OR "antibiotic" OR "antibiotics" OR "anti biotic" OR "anti biotics" OR "quinolone derivative" OR "Fluoroquinolones" OR "Fluoroquinolone" OR "Ciprofloxacin" OR "Enoxacin" OR "Enrofloxacin" OR "Fleroxacin" OR "Gatifloxacin" OR "Gemifloxacin" OR "Levofloxacin" OR "Moxifloxacin" OR "Norfloxacin" OR "Ofloxacin" OR "Pefloxacin" OR "Fosfomycin" OR "Fosfomycin" OR "Phosphomycin" OR "Phosphonomycin" OR "Monuril" OR "Cephalosporin Derivative" OR "Cephalosporins" OR "Cephalosporin" OR "Cefaclor" OR "Cefadroxil" OR "Cefamandole" OR "Cefatrizine" OR "Cefazolin" OR "Cefdinir" OR "Cefepime" OR "Cefixime" OR "Cefmenoxime" OR "Cefmetazole" OR "Cefonicid" OR "Cefoperazone" OR "Cefotaxime" OR "Cefotetan" OR "Cefotiam" OR "Cefoxitin" OR "Cefsulodin" OR "Ceftazidime" OR "Ceftibuten" OR "Ceftizoxime" OR "Ceftriaxone" OR "Cefuroxime" OR "Cephacetrile" OR "Cephalexin" OR "Cephaloglycin" OR "Cephaloridine" OR "Cephalothin" OR "Cephamycins" OR "Cephapirin" OR "Cephradine" OR "Carbapenems" OR "Carbapenems" OR "Carbapenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Imipenem" OR "Meropenem" OR "Aminoglycoside" OR "Aminoglycoside Antibiotic agent" OR "Gentamicin" OR "Tobramycin" OR "Aminoglycosides" OR "aminoglycoside" OR "gentamycin" OR "Gentamycins" OR "Gentamicin" OR "Gentamicins" OR "Sisomicin" OR "Netilmicin" OR "tobramycin" OR "Tobramycins" OR "cranberry extract" OR "cranberry" OR "cranberry juice" OR "Vaccinium macrocarpon" OR "cranberry" OR "cranberries" OR "cranberr*" OR "Methenamine" OR "Methenamine" OR "Hexamine" OR

"Hexamethylenetetramine" OR "Urotropin" OR "Aminoform" OR "Mannose" OR "Mannose" OR "D-Mannose" OR "Mannopyranoside" OR "Mannopyranose" OR "vaginal oestrogen" OR "vaginal oestrogens" OR "vaginal estrogen" OR "vaginal estrogens" OR (("intravaginal drug administration" OR "agents used intravaginally" OR "vaginal" OR "vagina" OR "intravaginal") AND ("Estrogen" OR "oestrogen" OR "oestrogens" OR "estrogen" OR "estrogens")) OR "drug therapy") OR AK=("Antibiotic agent" OR "Anti-Bacterial Agents" OR "Anti-Bacterial Agent" OR "Antibacterial Agents" OR "Antibacterial Agent" OR "antibiotic" OR "antibiotics" OR "anti biotic" OR "anti biotics" OR "quinolone derivative" OR "Fluoroquinolones" OR "Fluoroquinolone" OR "Ciprofloxacin" OR "Enoxacin" OR "Enrofloxacin" OR "Fleroxacin" OR "Gatifloxacin" OR "Gemifloxacin" OR "Levofloxacin" OR "Moxifloxacin" OR "Norfloxacin" OR "Ofloxacin" OR "Pefloxacin" OR "Fosfomycin" OR "Fosfomycin" OR "Phosphomycin" OR "Phosphonomycin" OR "Monuril" OR "Cephalosporin Derivative" OR "Cephalosporins" OR "Cephalosporin" OR "Cefaclor" OR "Cefadroxil" OR "Cefamandole" OR "Cefatrizine" OR "Cefazolin" OR "Cefdinir" OR "Cefepime" OR "Cefixime" OR "Cefmenoxime" OR "Cefmetazole" OR "Cefonicid" OR "Cefoperazone" OR "Cefotaxime" OR "Cefotetan" OR "Cefotiam" OR "Cefoxitin" OR "Cefsulodin" OR "Ceftazidime" OR "Ceftibuten" OR "Ceftizoxime" OR "Ceftriaxone" OR "Cefuroxime" OR "Cephacetrile" OR "Cephalexin" OR "Cephaloglycin" OR "Cephaloridine" OR "Cephalothin" OR "Cephamycins" OR "Cephapirin" OR "Cephradine" OR "Carbapenems" OR "Carbapenems" OR "Carbapenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Imipenem" OR "Meropenem" OR "Aminoglycoside" OR "Aminoglycoside Antibiotic agent" OR "Gentamicin" OR "Tobramycin" OR "Aminoglycosides" OR "aminoglycoside" OR "gentamycin" OR "Gentamycins" OR "Gentamicin" OR "Gentamicins" OR "Sisomicin" OR "Netilmicin" OR "tobramycin" OR "Tobramycins" OR "cranberry extract" OR "cranberry" OR "cranberry juice" OR "Vaccinium macrocarpon" OR "cranberry" OR "cranberries" OR "cranberr*" OR "Methenamine" OR "Methenamine" OR "Hexamine" OR "Hexamethylenetetramine" OR "Urotropin" OR "Aminoform" OR "Mannose" OR "Mannose" OR "D-Mannose" OR "Mannopyranoside" OR "Mannopyranose" OR "vaginal oestrogen" OR "vaginal oestrogens" OR "vaginal estrogen" OR "vaginal estrogens" OR (("intravaginal drug administration" OR "agents used intravaginally" OR "vaginal" OR "vagina" OR "intravaginal") AND ("Estrogen" OR "oestrogen" OR "oestrogens" OR "estrogen" OR "estrogens")) OR "drug therapy") OR AB=("Antibiotic agent" OR "Anti-Bacterial Agents" OR "Anti-Bacterial Agent" OR "Antibacterial Agents" OR "Antibacterial Agent" OR "antibiotic" OR "antibiotics" OR "anti biotic" OR "anti biotics" OR "quinolone derivative" OR "Fluoroquinolones" OR "Fluoroquinolone" OR "Ciprofloxacin" OR "Enoxacin" OR "Enrofloxacin" OR "Fleroxacin" OR "Gatifloxacin" OR "Gemifloxacin" OR "Levofloxacin" OR "Moxifloxacin" OR "Norfloxacin" OR "Ofloxacin" OR "Pefloxacin" OR "Fosfomycin" OR "Fosfomycin" OR "Phosphomycin" OR "Phosphonomycin" OR "Monuril" OR "Cephalosporin Derivative" OR "Cephalosporins" OR "Cephalosporin" OR "Cefaclor" OR "Cefadroxil" OR "Cefamandole" OR "Cefatrizine" OR "Cefazolin" OR "Cefdinir" OR "Cefepime" OR "Cefixime" OR "Cefmenoxime" OR "Cefmetazole" OR "Cefonicid" OR "Cefoperazone" OR "Cefotaxime" OR "Cefotetan" OR "Cefotiam" OR "Cefoxitin" OR "Cefsulodin" OR "Ceftazidime" OR "Ceftibuten" OR "Ceftizoxime" OR "Ceftriaxone" OR "Cefuroxime" OR "Cephacetrile" OR "Cephalexin" OR "Cephaloglycin" OR "Cephaloridine" OR "Cephalothin" OR "Cephamycins" OR "Cephapirin" OR "Cephradine" OR "Carbapenems" OR "Carbapenems" OR "Carbapenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Imipenem" OR "Meropenem" OR "Aminoglycoside" OR "Aminoglycoside Antibiotic agent" OR "Gentamicin" OR "Tobramycin" OR "Aminoglycosides" OR "aminoglycoside" OR "gentamycin" OR "Gentamycins" OR "Gentamicin" OR "Gentamicins" OR "Sisomicin" OR "Netilmicin" OR "tobramycin" OR "Tobramycins" OR "cranberry extract" OR

"cranberry" OR "cranberry juice" OR "Vaccinium macrocarpon" OR "cranberry" OR "cranberries" OR "cranberr*" OR "Methenamine" OR "Methenamine" OR "Hexamine" OR "Hexamethylenetetramine" OR "Urotropin" OR "Aminoform" OR "Mannose" OR "Mannose" OR "D-Mannose" OR "Mannopyranoside" OR "Mannopyranose" OR "vaginal oestrogen" OR "vaginal oestrogens" OR "vaginal estrogen" OR "vaginal estrogens" OR (("intravaginal drug administration" OR "agents used intravaginally" OR "vaginal" OR "vagina" OR "intravaginal") AND ("Estrogen" OR "oestrogen" OR "oestrogens" OR "estrogen" OR "estrogens")))) AND TI=("Urinary Tract Infection" OR "Urinary Tract Infection" OR "Urinary Tract Infections" OR "Urinary Infection" OR "Urinary Infections" OR "Urogenital Tract Infection" OR "Urogenital Tract Infections" OR "Urogenital Infection" OR "Urogenital Infections" OR "Pyuria" OR "Pyuria" OR "Pyurias" OR (("Cystitis" OR "Cystitis" OR "Pyelocystitis" OR "Pyelocystitis") NOT ("Interstitial Cystitis" OR "Interstitial Cystitis")) OR (("Pyelonephritis" OR "pyelonephritis") NOT ("Xanthogranulomatous Pyelonephritis" OR "Xanthogranulomatous Pyelonephritis")) OR "urosepsis" OR "urosepsis" OR "urinary sepsis" OR "urinary tract sepsis" OR "uroseptic") NOT (TI=(("Infant" OR "Child" OR "Adolescent" OR "Infant" OR "Infants" OR "Child" OR "Children" OR "Adolescent" OR "Adolescents" OR "pediatric" OR "paediatric" OR "pediatric*" OR "paediatric*") NOT ("Adult" OR "adult" OR "adults" OR "elderly")) OR AK=(("Infant" OR "Child" OR "Adolescent" OR "Infant" OR "Infants" OR "Child" OR "Children" OR "Adolescent" OR "Adolescents" OR "pediatric" OR "paediatric" OR "pediatric*" OR "paediatric*") NOT ("Adult" OR "adult" OR "adults" OR "elderly"))) AND py=(2017 OR 2018 OR 2019 OR 2020 OR 2021 OR 2022 OR 2023) AND (TI=("randomized controlled trial" OR "controlled clinical trial" OR "clinical trial" OR randomized OR randomised OR placebo OR randomly OR trial OR rct) OR AK=("randomized controlled trial" OR "controlled clinical trial" OR "clinical trial" OR randomized OR randomised OR placebo OR randomly OR trial OR rct) OR AB=("randomized controlled trial" OR "controlled clinical trial" OR "clinical trial" OR "randomized trial" OR "randomised trial" OR "placebo" OR "randomly" OR "rct")) NOT ti=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats") NOT TI=(("systematic reeview" OR "review") NOT "trial") NOT DT=(meeting abstract OR review OR editorial material))

Cochrane

https://www.cochranelibrary.com/advanced-search/search-manager

(("Antibiotic agent" OR "Anti Bacterial Agents" OR "Anti Bacterial Agent" OR "Antibacterial Agents" OR "Antibacterial Agent" OR "antibiotic" OR "antibiotics" OR "anti biotic" OR "anti biotics" OR "quinolone derivative" OR "Fluoroquinolones" OR "Fluoroquinolone" OR "Ciprofloxacin" OR "Enoxacin" OR "Enrofloxacin" OR "Fleroxacin" OR "Gatifloxacin" OR "Gemifloxacin" OR "Levofloxacin" OR "Moxifloxacin" OR "Norfloxacin" OR "Ofloxacin" OR "Pefloxacin" OR "Fosfomycin" OR "Fosfomycin" OR "Phosphomycin" OR "Phosphonomycin" OR "Monuril" OR "Cephalosporin Derivative" OR "Cephalosporins" OR "Cephalosporin" OR "Cefaclor" OR "Cefadroxil" OR "Cefamandole" OR "Cefatrizine" OR "Cefazolin" OR "Cefdinir" OR "Cefepime" OR "Cefixime" OR "Cefmenoxime" OR "Cefmetazole" OR "Cefonicid" OR "Cefoperazone" OR "Cefotaxime" OR "Cefotetan" OR "Cefotiam" OR "Cefoxitin" OR "Cefsulodin" OR "Ceftazidime" OR "Ceftibuten" OR "Ceftizoxime" OR "Ceftriaxone" OR "Cefuroxime" OR "Cephacetrile" OR "Cephalexin" OR "Cephaloglycin" OR "Cephaloridine" OR "Cephalothin" OR "Cephamycins" OR "Cephapirin" OR "Cephradine" OR "Carbapenems" OR "Carbapenems" OR "Carbapenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR

"Imipenem" OR "Meropenem" OR "Aminoglycoside" OR "Aminoglycoside Antibiotic agent" OR "Gentamicin" OR "Tobramycin" OR "Aminoglycosides" OR "aminoglycoside" OR "gentamycin" OR "Gentamycins" OR "Gentamicin" OR "Gentamicins" OR "Sisomicin" OR "Netilmicin" OR "tobramycin" OR "Tobramycins" OR "cranberry extract" OR "cranberry" OR "cranberry juice" OR "Vaccinium macrocarpon" OR "cranberry" OR "cranberries" OR "cranberr*" OR "Methenamine" OR "Methenamine" OR "Hexamine" OR "Hexamethylenetetramine" OR "Urotropin" OR "Aminoform" OR "Mannose" OR "Mannose" OR "D Mannose" OR "Mannopyranoside" OR "Mannopyranose" OR "vaginal oestrogen" OR "vaginal oestrogens" OR "vaginal estrogen" OR "vaginal estrogens" OR (("intravaginal drug administration" OR "agents used intravaginally" OR "vaginal" OR "vagina" OR "intravaginal") AND ("Estrogen" OR "oestrogen" OR "oestrogens" OR "estrogen" OR "estrogens")) OR "drug therapy"):ti,ab,kw AND ("Urinary Tract Infection" OR "Urinary Tract Infection" OR "Urinary Tract Infections" OR "Urinary Infection" OR "Urinary Infections" OR "Urogenital Tract Infection" OR "Urogenital Tract Infections" OR "Urogenital Infection" OR "Urogenital Infections" OR "Pyuria" OR "Pyuria" OR "Pyurias" OR (("Cystitis" OR "Cystitis" OR "Pyelocystitis" OR "Pyelocystitis") NOT ("Interstitial Cystitis" OR "Interstitial Cystitis")) OR (("Pyelonephritis" OR "pyelonephritis") NOT ("Xanthogranulomatous Pyelonephritis" OR "Xanthogranulomatous Pyelonephritis")) OR "urosepsis" OR "urosepsis" OR "urinary sepsis" OR "urinary tract sepsis" OR "uroseptic"):ti NOT (("Infant" OR "Child" OR "Adolescent" OR "Infant" OR "Infants" OR "Child" OR "Children" OR "Adolescent" OR "Adolescents" OR "pediatric" OR "paediatric" OR "pediatric*" OR "paediatric*") NOT ("Adult" OR "adult" OR "adults" OR "elderly")):ti)

AND py=(2017 OR 2018 OR 2019 OR 2020 OR 2021 OR 2022 OR 2023)

NOT DT=(meeting abstract))

Supplementary Table 1: Overview of included studies.

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Aloush 2019	Experimental (n = 171) Hospital	Therapeutic – oral antimicrobial	Both women and men	UTI – phenotype not specified	Yes, CDC guideline	Required	–	–	Not required	Required, cut-off 10^5 CFU/mL
Arakawa 2019	Experimental (n = 115) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Multiple types of UTI including complicated UTI	No	Required	Regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Required, > 10 leukocytes/µl	Required, cut-off 10^5 CFU/mL
Babar 2021	Experimental (n = 145) Primary care or outpatient	Prophylactic – cranberry	Women	UTI – phenotype not specified	Yes, IDSA clinical practice guideline	Required	–	–	Not required	Not required
Boel 2020	Observational (n = 1129) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Bacteraemic / febrile UTI	No	Not required	–	–	Not required	Required, cut-off 10^3 CFU/mL
Botros 2021	Experimental (n = 92) Primary care or outpatient	Prophylactic – methenamine	Women	UTI – phenotype not specified	Yes, EAU guideline	Required	–	–	Not required	Required, cut-off 10^3 CFU/mL

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Bruyère 2019	Experimental (n = 85) Primary care or outpatient	Prophylactic – cranberry	Women	UTI – phenotype not specified	No	Required	–	–	Not required	Required, cut-off 10^5 CFU/mL
Costache 2019	Experimental (n = 40) Primary care or outpatient	Therapeutic – other	Both women and men	Acute cystitis	No	Required	–	–	Not required	Required, cut-off 10^3 CFU/mL
Diebold 2021	Experimental (n = 78) Primary care or outpatient	Prophylactic – other	Women	Acute cystitis	No	Required	–	–	Not required	Not required
Drekonja 2021	Experimental (n = 272) Primary care or outpatient	Therapeutic – oral antimicrobial	Men	Acute cystitis	No	Required	–	–	Not required	Not required
Eckburg 2022	Experimental (n = 1372) Hospital	Therapeutic – oral antimicrobial	Both women and men	Complicated UTI	Yes, FDA guideline	Required	Not regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Required, either positive leukocyte esterase or > 10 leukocytes per hpf or mm^3	Not required

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Edlund 2022	Experimental (n = 152) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	No	Required	Not regarded as subset of complicated UTI, and defined separately	Based on host factors only	Required, positive leukocyte esterase	Not required
El Nekidy 2021	Observational (n = 85) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	UTI - phenotype not specified	No	Required	-	-	Not required	Required, no cut-off specified
Ferrante 2019	Experimental (n = 35) Primary care or outpatient	Prophylactic – other	Women	UTI - phenotype not specified	No	Required	-	-	Not required	Required, no cut-off specified
Gama 2020	Experimental (n = 272) Primary care or outpatient	Prophylactic – methenamine	Both women and men	UTI - phenotype not specified	No	Required	-	-	Not required	Not required
Gamble 2022	Observational (n = 153) Hospital	Therapeutic – oral antimicrobial	Both women and men	Multiple types of UTI including complicated UTI	Yes, EAU guideline	Not required	Not regarded as subset of complicated UTI, and defined separately	Based on host factors only	Not required	Required, no cut-off specified

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Gágyor 2021	Experimental (n = 398) Primary care or outpatient	Prophylactic – other	Women	Acute cystitis	No	Required	-	-	Not required	Not required
Harding 2022	Experimental (n = 240) Primary care or outpatient	Prophylactic – methenamine	Women	UTI - phenotype not specified	Yes, Public Health England (clinical practice guideline)	Required	-	-	Not required	Not required
Jansaker 2019	Experimental (n = 368) Primary care or outpatient	Therapeutic – oral antimicrobial	Women	Acute cystitis	No	Required			Not required	Not required
Kaye 2019	Experimental (n = 456) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	No	Required	Not regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Required, either positive leukocyte esterase or > 10 leukocytes per hpf	Not required

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Kohno 2021	Experimental (n = 83) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	No	Required	Regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Required, either positive leukocyte esterase or > 10 leukocytes per hpf or mm^3	Required, cut-off 10^5 CFU/mL
Koradia 2019	Experimental (n = 81) Primary care or outpatient	Prophylactic – cranberry	Women	Acute cystitis	Yes, EAU guideline	Required	-	-	Not required	Required, cut-off 10^3 CFU/mL
Li 2021	Experimental (n = 208) Primary care or outpatient	Therapeutic – oral antimicrobial	Both women and men	Multiple types of UTI including complicated UTI	No	Required	Only complicated UTI is investigated, acute pyelonephritis is not mentioned	Based on both host factors and systemic involvement	Required, > 10 leukocytes/µl or > 5 per hpf	Required, cut-off 10^5 CFU/mL

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Lojanapiwat 2019	Experimental (n = 289) Hospital	Therapeutic – oral antimicrobial	Both women and men	Complicated UTI	No	Required	Regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Conditionally required	Not required
Mir 2019	Experimental (n = 230) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	Yes, FDA guideline	Required	Regarded as subset of complicated UTI, but not defined separately	Based on both host factors and systemic involvement	Not required	Required, cut-off 10^5 CFU/mL
Mirzaei 2019	Experimental (n = 30) Primary care or outpatient	Prophylactic – other	Women	Acute cystitis	No	Required	-	-	Not required	Required, cut-off 10^3 CFU/mL
Montelin 2019	Observational (n = 171) Primary care or outpatient	Therapeutic – oral antimicrobial	Men	Acute cystitis	No	Required	-	-	Not required	Required, cut-off 10^3 CFU/mL

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Nestler 2021	Experimental (n = 173) Primary care or outpatient	Prophylactic – other	Women	UTI - phenotype not specified	No	Required	-	-	Not required	Required, cut-off 10^3 CFU/mL
Overcash 2020	Experimental (n = 22) Hospital	Therapeutic – oral antimicrobial	Women	Acute cystitis	No	Required	-	-	Conditionally required, either positive leukocyte esterase or at least 10 leukocytes/ mm^3	Not required
Overcash 2019	Experimental (n = 31) Hospital	Therapeutic – oral antimicrobial	Women	Acute cystitis	Yes, FDA guideline	Required	-	-	Required, positive leukocyte esterase	Not required
Pierotti 2020	Observational (n = 306) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Bacteraemic / febrile UTI	No	Not required	-	-	Not required	Not required
Radulescu 2020	Experimental (n = 120) Primary care or outpatient	Prophylactic – cranberry	Women	Acute cystitis	Yes, EAU guideline	Required	-	-	Not required	Not required

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Ryanto 2019	Observational (n = 152) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Multiple types of UTI including complicated UTI	No	Not required	Acute pyelonephritis is mentioned, but not defined	Complicated UTI is mentioned, but not defined	Not required	Not required
Safwat 2019	Experimental (n = 389) Primary care or outpatient	Prophylactic – other	Men	UTI – phenotype not specified	No	Not required	–	–	Not required	Required, no cut-off specified
Sagan 2020	Experimental (n = 80) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	No	Required	Not regarded as subset of complicated UTI, and defined separately	Based on host factors only	Required, either positive leukocyte esterase or > 10 leukocytes per mm^3 or hpf	Not required
Sashidhar 2022	Experimental (n = 50) Primary care or outpatient	Therapeutic – oral antimicrobial	Both women and men	Acute cystitis	No	Required	–	–	Required, method not specified	Not required
Senard 2020	Observational (n = 50) Hospital	Therapeutic – intravenous antimicrobial	Men	Bacteraemic / febrile UTI	Yes, French clinical practice guideline	Required	–	–	Not required	Required, no cut-off specified

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Sharara 2020	Observational (n = 186) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Acute pyelonephritis	No	Required	Defined separately, not mentioned whether part of complicated UTI	-	Required, > 10 leukocytes per hpf	Required, cut-off 50000 CFU/mL
Sojo-Dorado 2022	Experimental (n = 143) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Bacteraemic / febrile UTI	No	Conditionally required	-	-	Conditionally required, either positive leukocyte esterase or > 10 leukocytes/mm^3	Required, no cut-off specified
Sorli 2019	Observational (n = 33) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Multiple types of UTI, including acute pyelonephritis	Yes, CDC guideline	Required	Defined separately, not mentioned whether part of complicated UTI	-	Not required	Required, cut-off 10^5 CFU/mL

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Stalenhoef 2019	Experimental (n = 63) Primary care or outpatient	Prophylactic – antimicrobial	Both women and men	UTI – phenotype not specified	No	Required	-	-	Required, method not specified	Required, cut-off 10^3 CFU/mL
Tehrani 2021	Experimental (n = 59) Hospital	Therapeutic – oral antimicrobial	Both women and men	Acute pyelonephritis	No	Required	Defined separately, not mentioned whether part of complicated UTI	-	Required, > 10 leukocytes per hpf	Not required
Ten Doesschate 2019	Observational (n = 40) Primary care or outpatient	Therapeutic – oral antimicrobial	Both women and men	Multiple types of UTI including bacteraemic / febrile UTI	No	Required	-	-	Not required	Required, cut-off 10^4 CFU/mL
Ten Doesschate 2021	Experimental (n = 97) Hospital	Therapeutic – oral antimicrobial	Women	Complicated UTI	No	Required	Regarded as subset of complicated UTI, but not defined separately	Febrile UTI is considered a complicated UTI	Not required	Required, cut-off 10^4 CFU/mL

Supplementary Table 1: Continued

Study	Design & setting	Intervention	Population	Type of UTI	Refers to guideline	Symptoms	Acute pyelonephritis	Complicated UTI	Pyuria	Positive urine culture & cut-off
Tseng 2020	Experimental (n = 26) Primary care or outpatient	Prophylactic – other	Women	UTI – phenotype not specified	No	Required	–	–	Conditionally required, either positive leukocyte esterase or > 5 cells/hpf	Conditionally required, cut-off 10^4 CFU/mL
Tullos 2020	Observational (n = 180) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	UTI – phenotype not specified	No	Not required	–	–	Not required	Required, no cut-off specified
Wagenlehner 2019	Experimental (n = 609) Hospital	Therapeutic – intravenous antimicrobial	Both women and men	Complicated UTI	Yes, FDA guideline	Required	Regarded as subset of complicated UTI, and defined separately	Based on both host factors and systemic involvement	Required, either positive leukocyte esterase or > 10 leukocytes per hpf	Not required
Wald-Dickler 2022	Observational (n = 322) Hospital	Therapeutic – oral antimicrobial	Both women and men	Complicated UTI	No	Required	Regarded as subset of complicated UTI, but not defined separately	Based on both host factors and systemic involvement	Not required	Required, no cut-off specified

Abbreviations: UTI = urinary tract infection, CFU = colony-forming units; CDC = Centers for Disease Control and Prevention; IDSA = Infectious Diseases Society of America; EAU = European Association of Urology; FDA = Food and Drug Administration; hpf = high-power field.

Chapter 3

A reference standard for urinary tract infection research: a multidisciplinary Delphi consensus study

Manu P. Bilsen, Simon P. Conroy, Caroline Schneeberger, Tamara N. Platteel, Cees van Nieuwkoop, Lona Mody, Jeffrey M. Caterino, Suzanne E. Geerlings, Béla Köves, Florian Wagenlehner, Marleen Kunneman, Leo G. Visser, Merel M.C. Lambregts, and members of the UTI reference standard consensus group

Lancet Infect Dis. 2024 Mar 5:S1473-3099(23)00778-8

Abstract

The absence of a consensus-based reference standard for urinary tract infection (UTI) research adversely affects the internal and external validity of diagnostic and therapeutic studies. This hinders the accumulation of evidence for a disease that imposes a significant burden on patients and society, particularly in an era of increasing antimicrobial resistance. We conducted a three-round Delphi study involving an international, multidisciplinary panel of UTI experts (n = 46), and achieved a high degree of consensus (94%) on the final reference standard. New-onset dysuria, frequency and urgency were considered major symptoms, and non-specific symptoms in older patients were not deemed indicative of UTI. The reference standard distinguishes between UTI with and without systemic involvement, abandoning the term 'complicated UTI'. Moreover, different levels of pyuria were incorporated in the reference standard, encouraging quantification of pyuria in studies conducted in all healthcare settings. The traditional bacteriuria threshold (10^5 colony-forming units (CFU)/mL) was lowered to 10^4 CFU/mL. This new reference standard can be used for UTI research across many patient populations and has the potential to increase homogeneity between studies.

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in the community. [1] Its high incidence and recurrence rate lead to a decreased quality of life, excessive healthcare costs, and significant use of antimicrobials. [1, 2] UTI diagnosis is commonly based on a combination of symptoms and signs, pyuria, and culture results. Current UTI research primarily focuses on improving diagnostics and developing novel therapeutic and prophylactic modalities, such as new antimicrobials and vaccines. [3, 4] However, UTI studies are impeded by the lack of a consensus-based reference standard for UTI. The absence of a reference standard has several consequences. Firstly, it introduces bias into estimates of diagnostic accuracy and efficacy (also known as verification bias), affecting the internal validity of a study. [5] Secondly, if different criteria are used across studies, results cannot be readily compared, compromising the external validity of a study. These drawbacks are particularly relevant in the context of growing antimicrobial resistance, in which reliable efficacy and safety data on novel antimicrobials for UTI are crucial. Moreover, from an ethical standpoint, it is vital to ensure consistent treatment of study participants and patients, as well as accurate reporting of study findings.

Although several proposed definitions exist, they are limited in their ability to be used in the majority of UTI studies. Centers for Disease Control and Prevention guidelines were primarily formulated for surveillance of nosocomial and catheter-associated UTI, and the revised McGeer criteria were designed for studies in long-term care facilities, limiting their applicability. [6, 7] The European Medicines Agency (EMA) and Food and Drug Administration (FDA) have published guidelines for the development and approval of drugs for the treatment of uncomplicated and complicated UTI, including acute pyelonephritis. [8-10] However, these guidelines apply different symptom criteria, and definitions of complicated UTI are not uniform. Moreover, the EMA guideline does not specify a minimum number of symptoms, and the FDA guideline does not provide a pyuria threshold for uncomplicated UTI, leaving room for interpretation. Furthermore, it is unclear which research methodology was employed in the development of these guidelines. Prior to this study, we performed a systematic review evaluating recently published UTI studies, which demonstrated low adherence to FDA and EMA guidelines. [11] Researchers more frequently defined UTI based on their own criteria or clinical practice guidelines, leading to heterogeneous UTI definitions across studies. These findings underscore the necessity for a multidisciplinary-

supported reference standard for UTI, developed specifically for research purposes. Consequently, the primary aim of this study was to achieve consensus on a reference standard for UTI, applicable to adult women and men, including older patients, who participate in studies focusing on bacterial UTI, excluding those related to indwelling catheters.

Methods

Study design

In order to gain consensus on a reference standard, a Delphi study was conducted and reported following CREDES recommendations. [12] The Delphi method has four main characteristics: an expert panel is questioned about the issue of interest, the process is anonymous to reduce the effect of dominant personalities, the questionnaires are iterative in nature, and the design of the subsequent rounds is informed by a summary of the group response of the previous round. [13] The Delphi method was chosen over other consensus methods (e.g. the nominal group technique) because it offers the advantage of not being limited by geographical and temporal constraints. [14] We planned a minimum of three rounds, with the possibility of additional rounds, depending on the level of consensus. Data was collected using REDCap. [15] An overview of the study design is provided in the Supplementary Material (see **Supplementary Figure 1**), which will be discussed in detail below. This study was registered at ClinicalTrials.gov (ID NCT05365906).

Core group and expert panel

Based on their publication record and clinical expertise, UTI experts were invited by the principal investigators (M.P.B., S.P.C., M.M.C.L.) to be part of the research team, henceforth described as the core group. All core group members who were contacted (via email) agreed to participate. As the primary users of the research reference standard will include researchers from multiple specialties and countries, we ensured multidisciplinary and multinational representation in the core group. The core group consisted of 11 experts from the following countries: the Netherlands (n = 6), the United States (n = 2), the United Kingdom (n = 1), Germany (n = 1), and Hungary (n = 1) and a moderator (M.P.B.). Primary specialties represented in the core group were infectious diseases (n = 4), geriatric medicine (n = 2), urology (n = 2), primary care (n = 1), emergency medicine (n = 1), and microbiology (n = 1); some experts also had secondary specialties. Since the core group members were tasked with designing and interpreting the questionnaire

rounds, as well as constructing the reference standard, a separate expert panel was invited to participate in the Delphi questionnaire and feedback rounds. The core group proposed experts from their respective specialties, and geographical and gender equity were encouraged. There were no specific exclusion criteria for expert panellists. Experts were invited through an email containing an explanation of study objectives, the required effort, outputs, and rewards (an acknowledgement of study participation at publication). The identities of the expert panellists who participated were known exclusively to the core group. Consent to participate in the Delphi surveys was assumed if the surveys were completed and returned. Expert panellists could withdraw at any time.

Expert panel size

In the literature, Delphi panel size varies between ten to several hundred participants. [13] Small panels may not provide a representative range of judgments on the topic at hand, while large panels may lead to low response rates and a significant amount of missing data. In case of a homogenous background of Delphi panellists, around ten to fifteen subjects are usually sufficient. [16] Given the multidisciplinary nature of our expert panel, we aimed to include a minimum of 40 expert panel participants.

Delphi round 1 (R1)

Based on signs, symptoms, and diagnostic tests listed in two previous studies, the core group prepared a questionnaire for the expert panel containing 48 items (see **Supplementary Figure 2**). [11, 17] We clarified the purpose of the questionnaire and structured it into five categories: signs and symptoms (20 items), urinalysis (six items), microbiology (ten items), items focused on ruling out UTI (five items), and items addressing systemic involvement (seven items). We used the RAND/UCLA Appropriateness Method [18] to determine the expert panel's assessment of the degree to which each item indicated UTI, using a Likert scale ranging from 1 (‘not at all indicative’) to 9 (‘very indicative’). An item was deemed (1) indicative of UTI in case of a panel median ≥ 6·5, without disagreement, (2) not indicative of UTI in case of a panel median of ≤ 3·5, without disagreement, and (3) uncertain if the panel median lay in between indicative and not indicative, or any median with disagreement. Disagreement was considered to occur if both extremes of the Likert scale (1–3 and 7–9) contained more than a third of responses. [18] If disagreement occurred in > 20% of items, we planned to repeat this questionnaire round for

the items that met disagreement criteria, after which no further iterations were planned, as R1 primarily served to facilitate the core group in constructing the reference standard and differences in perspectives concerning the topic were considered valuable input.

The questionnaire explicitly stated that signs and symptoms should be graded based on recent onset, and that items should be graded for UTI in general, unless a specific patient population or anatomic site (i.e. cystitis or pyelonephritis) was mentioned. In the signs/symptoms, urinalysis, and microbiology categories, we included additional questions to inquire whether experts would modify their ratings based on the sex (assigned at birth) and age (≥ 65 years) of the patient in question. Per category, experts were given the opportunity to provide extra comments justifying their grading, but they could not add new items. Moreover, we collected data on specialty, country of practice and years working in the field post-training. This questionnaire was pilot tested for content and clarity by three independent infectious diseases specialists.

Development of reference standard and case vignettes

Median scores and expert panel comments (organised thematically by their content) were presented to the core group in an online meeting in June 2022. Based on R1 results and available literature, a reference standard was drafted by the principal investigators. A scoring system was incorporated into the reference standard to reflect that each individual item carried a different weight in its contribution to UTI diagnosis. This draft version was then discussed with all members of the core group in two additional online meetings in July 2022. All core group members participated in at least one online meeting to provide their input for the development of the reference standard. Minutes of group discussions and adjustments to the reference standard were sent to core group members so that additional comments could be provided via email. Disagreements were resolved through discussion and a draft version of the reference standard had to be agreed upon by all core group members before initiation of Delphi round 2 (R2). Rather than solely assessing consensus on the reference standard through expert panel grading in R2 and Delphi round 3 (R3), alignment between the reference standard (scoring system) and the expert panel's interpretation of a set of case vignettes was evaluated. The core group designed ten case vignettes, incorporating various combinations of lower urinary tract and systemic signs and symptoms, pyuria, and urine culture results. The case vignettes included different age groups, sexes,

and health care settings. Cases could be graded as 'definite UTI', 'probable UTI', 'possible UTI', or 'no UTI', analogous to the four UTI categories of the reference standard (based on the scoring system). These categories were chosen to reflect the degrees of certainty in the diagnosis of UTI. To ensure clarity and proper wording, case descriptions were pilot tested by three independent physicians.

Delphi round 2 and 3 (R2 and R3)

In R2, the expert panel first graded the case vignettes, and for each case, experts were given the opportunity to justify their grading. Next, a draft version of the reference standard was presented to the expert panel. Per domain of the reference standard (symptoms and signs, systemic criteria, pyuria, and culture results), experts could indicate their agreement or disagreement with a 'yes' or 'no' answer. In case of disagreement, experts were requested to provide a rationale. Furthermore, overall agreement with the reference standard was assessed through a five-point Likert scale ranging from 1 ('strongly disagree') to 5 ('strongly agree'), and additional comments were encouraged. R2 results were discussed in two online core group meetings in September and October 2022. Based on these results and an additional literature review, adjustments were made to the reference standard. Adjustments had to be agreed upon by all core group members before R3 could be initiated. In R3, a summary of the expert panel grading from R2 was presented, and experts were asked to regrade the same ten case vignettes. Subsequently, the experts regraded the adjusted reference standard, which was presented alongside a description of how the expert panel comments had been addressed. Consensus was defined a priori as a minimum of 80% of experts voting 'agree' or 'strongly agree' and none of the experts voting 'disagree'. If consensus was not reached after R3, subsequent rounds were planned until consensus was reached.

Results

Of the 62 experts who were invited to be a part of the expert panel, 46 (74%) agreed to partake. Two experts declined participation due to either retirement or time constraints, but both suggested alternates. Reasons for non-participation of the other invited experts were unknown. Expert panel characteristics are detailed in **Table 1**. Experts were located in various countries in Europe and North America and had been practicing as a specialist for a median of 13 years (IQR 8 – 20). Three Delphi questionnaire rounds were conducted between April 2022 and December 2022. Response rates were 100%, 87%, and 80% for R1, R2, and R3, respectively.

Complete questionnaires for all three rounds can be found in the Supplementary Material.

Table 1: Expert panel characteristics.

Expert panel characteristics	n = 46
Primary specialty n (%)	
Infectious diseases	13 (28)
Urology	9 (20)
Microbiology	7 (15)
Geriatrics	6 (13)
Family medicine	6 (13)
Emergency medicine	5 (11)
Country of practice n (%)	
United States	14 (30)
The Netherlands	13 (28)
Germany	5 (11)
United Kingdom	3 (7)
Sweden	3 (7)
Belgium	3 (7)
Norway	2 (4)
Canada	1 (2)
Spain	1 (2)
Switzerland	1 (2)
Years working in the field post-training median (IQR)	13 (8 – 20)

One expert panellist was a primary care physician in training but had extensive research and clinical UTI experience and was thus included in the expert panel. Three of the included experts had secondary specialties: general surgery (n = 1), epidemiology (n = 1) and general internal medicine (n = 1).

Delphi round 1

None of the 48 items in R1 met our predefined disagreement criterion. As such, this round was not repeated. Median expert panel ratings and respective interquartile ranges are shown in **Supplementary Figure 2**. In total, 19 of 48 items (40%) were deemed indicative of UTI, 9 of 48 items (19%) were rated 'not indicative', and 20 of 48 items (42%) were of uncertain value. Regarding symptoms and signs, new-onset dysuria, urgency, frequency and symptom recognition (i.e. patient recognises symptoms as UTI) were voted most indicative of UTI, with a high degree of consensus (IQR ≤ 2). Twenty-one of 46 experts (46%) would change their grading if it concerned an older patient, for which the most cited reasons

were: altered symptom presentation (e.g. a higher rate of non-specific symptoms such as delirium and malaise) (n = 11), and decreased specificity of lower urinary tract symptoms due to pre-existing symptoms (n = 3). Thirty-six experts (78%) would not change their grading for male patients.

All pyuria and nitrite items related to older patients were deemed less indicative of UTI than for younger patients, although experts added that their grading would primarily depend on symptom presentation (n = 7), and quality of the urine sample (n = 4). For microbiology items, isolation of the same pathogen from blood and urine cultures received the highest panel median. Regarding the colony-forming units per mL (CFU/mL) threshold for significant bacteriuria, ≥ 10^4 CFU/mL was considered indicative of UTI. Half of the experts who provided additional comments suggested lower (10^2 to 10^4) thresholds for CFU/mL, particularly if *Escherichia coli* was isolated. Seventeen experts (37%) would lower the threshold for urine samples obtained through single 'in-out' urinary catheterisation. Moreover, median scores for items ruling out UTI were highest for the absence of symptoms (in cystitis), pyuria or bacteriuria (without pretreatment). All systemic items other than hypothermia were graded to be useful for differentiating upper from lower UTI, although their low specificity was noted.

Delphi round 2 and adjustments to reference standard

Case vignette results and expert panel comments to each case are shown in the Supplementary Material (see **Supplementary Tables 1 and 2**). For all ten cases, the majority vote aligned with the UTI category as determined by the reference standard. Overall agreement with the drafted reference standard in R2 was 78% (29/37) (see **Supplementary Figure 3**). Per domain, agreement was 82% for symptoms and signs, 70% for systemic criteria, and 68% each for pyuria and culture results. Based on expert panel feedback several changes were made to the reference standard after R2. In the symptoms and signs domain, suprapubic pain, perineal pain (or prostate tenderness on examination) and flank pain (or costovertebral angle tenderness) were moved from major to minor symptoms. Moreover, the option of two minor symptoms was added to the 2-point category. In the systemic criteria domain, an elevated white blood cell (WBC) count was added as a criterion and the C-reactive protein (CRP) cut-off was lowered. Leukocyte esterase was removed from the pyuria domain and new units (cells per high-power field) were added. In the culture domain, the CFU/mL threshold for *Escherichia coli* (10^2 CFU/mL) was adjusted to 10^3, the maximum number of

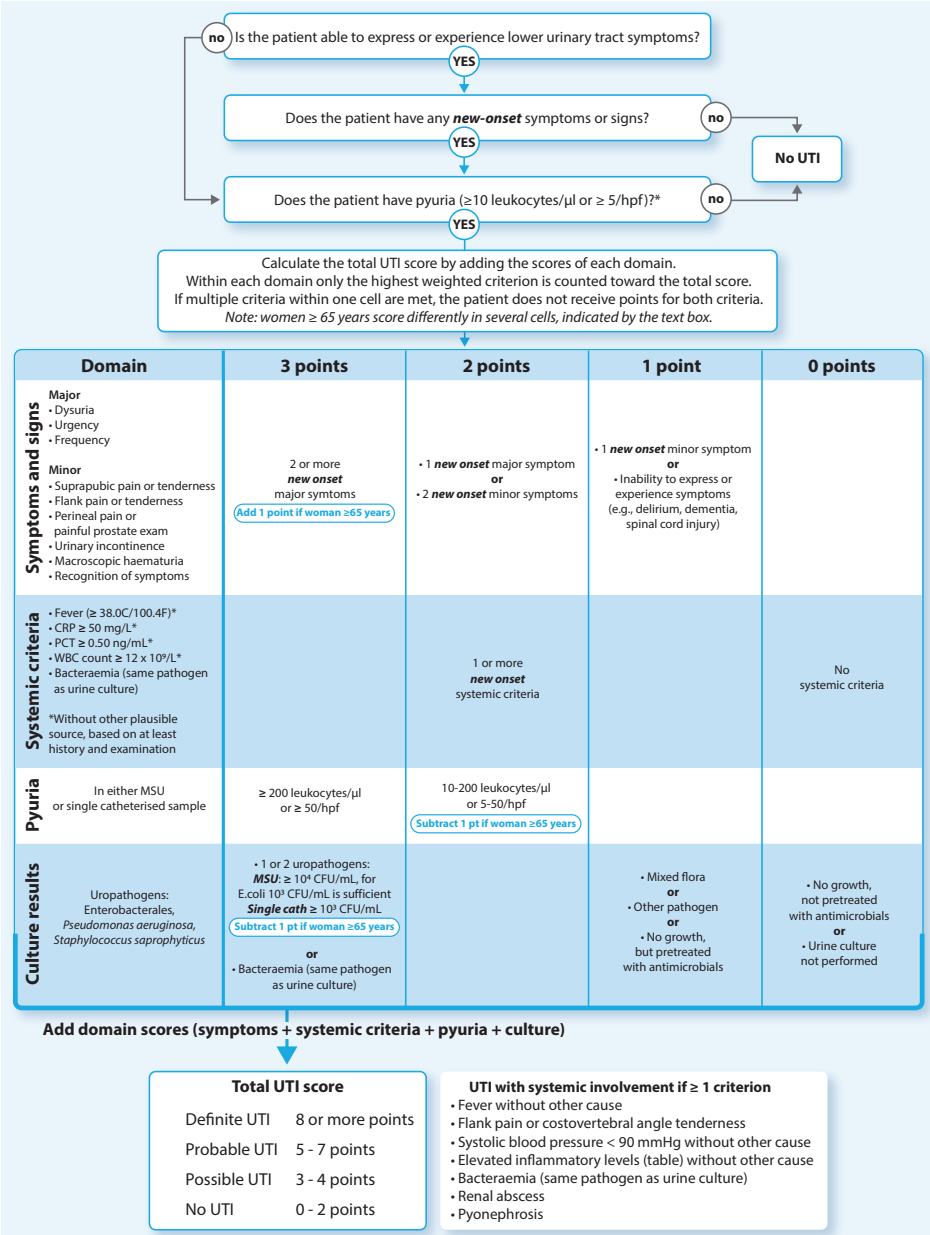


Figure 1: Research reference standard for urinary tract infections. * Pyuria must be quantified, a leukocyte esterase result (urine dipstick) is insufficient. In case of obstructive uropathy or absolute neutropenia, pyuria may be absent and the total UTI score may be calculated. Abbreviations: UTI = urinary tract infection, CRP = C-reactive protein, PCT = procalcitonin, WBC = white blood cell, MSU = midstream urine, CFU = colony-forming units, hpf = high-power field.

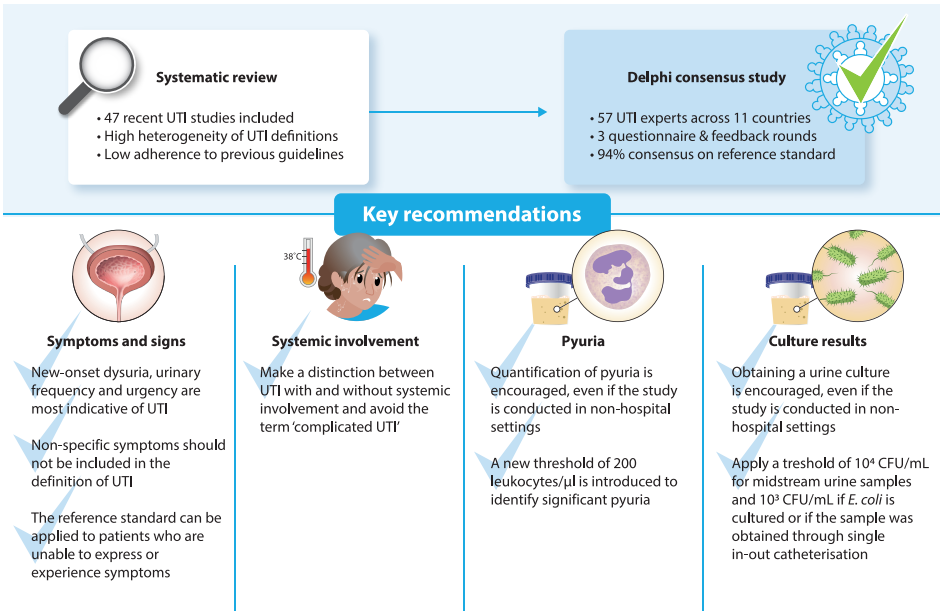


Figure 2: Summary of study findings. Abbreviations: UTI = urinary tract infection, CFU = colony-forming units

uropathogens in the 3-point category was increased to two, nitrites were removed, and *Staphylococcus aureus* was removed from the list of typical uropathogens. Final UTI score categories remained the same.

Delphi round 3

Displaying expert panel interpretation of the case vignettes from R2 led to an increased level of agreement among experts for all ten cases in R3, as shown in **Supplementary Table 1**. Consensus was reached regarding the adjusted reference standard, with 31/33 experts (94%) either agreeing or strongly agreeing with it, while no one disagreed (**Supplementary Figure 3**). The final reference standard is presented in **Figure 1** and key recommendations are summarised in **Figure 2**.

Discussion

In this international Delphi study, we systematically addressed all issues relating to UTI diagnosis and nomenclature and achieved consensus on a reference standard designed specifically for research purposes. By including a broad range

of stakeholders, we incorporated viewpoints from different medical specialties to increase applicability and endorsement across major specialties that frequently encounter UTI.

Signs and symptoms

In the symptoms and signs domain, dysuria, urgency and frequency were chosen as major symptoms, as these symptoms received the highest median scores in R1. This decision was supported by findings from a systematic review showing that these symptoms were most often used in study definitions for UTI. [11] Given that co-occurrence of two lower urinary tract symptoms increases the likelihood of UTI, and these symptoms are not 100% specific for UTI if present alone (e.g. overactive bladder, genitourinary syndrome of menopause), the core group decided to award most points if two or more major symptoms were present. [19] The value of symptom recognition was most debated, as some experts feared that (older) patients would wrongfully attribute symptoms to a UTI based on prior misdiagnosis. However, based on a high median score in R1 and findings by Gupta et al. [20] showing that premenopausal women can accurately self-diagnose UTI, symptom recognition was left in as a minor criterion. Although some expert panellists commented that older patients more frequently present with non-specific symptoms, all non-specific symptoms in R1 received low median scores. This finding is in line with the clinical decision tool for suspected UTI in frail older adults developed through a consensus study by van Buul et al. [17], in which non-specific symptoms, regardless of urinalysis results, do not warrant empirical antimicrobial treatment. Furthermore, another Delphi study, which specifically addressed diagnostic stewardship in the context of ordering urine cultures, classified these nonspecific symptoms as inappropriate justifications for requesting such cultures. [21] The core group believed that older adults who are unable to reliably communicate symptoms (e.g. due to delirium or dementia) should not be excluded from the reference standard, as this population is disproportionately affected by UTI, and a reference standard is vital for research in this population. Considering R1 results and the high background prevalence of asymptomatic pyuria and bacteriuria in this population (especially in women ≥ 65 years), the core group decided to deduct points in pyuria and culture domains for women in this age group. [22-24] Consequently, an older woman with pyuria and bacteriuria, who is unable to communicate symptoms, can only achieve a

classification of 'possible UTI' at best. To offset this deduction, women ≥ 65 years with two major symptoms are granted an additional point.

Systemic criteria

Regarding systemic criteria, core group discussions and expert panel comments focused on the available evidence and cut-off values of the included inflammatory parameters (CRP ≥ 50 mg/L, procalcitonin ≥ 0·50 ng/mL and WBC count ≥ 12 x 10^9/L). Although inflammatory parameter levels are dynamic and depend on the moment of measurement, and thresholds are chosen based on whether high specificity or sensitivity is preferred, the core group felt it was important to provide cut-off values to ensure uniformity. Acknowledging the limited evidence for the included inflammatory parameters regarding UTI with systemic involvement, we chose cut-offs by extrapolating data from studies investigating UTI-related bloodstream infection (BSI) and sepsis. Procalcitonin ≥ 0·50 ng/mL had a sensitivity of 82% and specificity of 66% for BSI in a study with 581 adults with febrile UTI. [25] In a recently published cohort study containing a subset of nearly 15000 adults with presumed UTI, procalcitonin ≥ 0·50 ng/mL showed a sensitivity of 78% and a specificity of 61% for BSI. [26] In an emergency department study involving 160 patients with acute pyelonephritis, sensitivity and specificity of WBC count > 12 x 10^9/L (threshold used in the Surviving Sepsis Campaign guideline) and CRP > 40 mg/L were 58% and 82%, and 76% and 95%, respectively. [27] To further increase specificity, we state that no other plausible source must be present, based on at least history and examination. The core group decided to abandon the term 'complicated UTI' and instead to make a distinction between UTI with and without systemic involvement. We recently showed that 'complicated UTI' definitions are heterogeneous (based on both host factors and systemic involvement), which leads to disparities between studies and hampers the interpretation of their results for different clinical phenotypes. [11] A distinction based solely on clinical phenotype would align more with clinical practice and would facilitate UTI studies evaluating new antimicrobials to include only patients from the target population.

Pyuria

Given that the absence of pyuria, when quantified, rules out UTI (at least in symptomatic women with confirmed bacteriuria) and expert panel grading in R1, the core group agreed that pyuria, albeit with a low threshold, should be an 'entry

criterion' of the reference standard. [28, 29] An exception to this pyuria rule was made for patients with complete obstructive uropathy or absolute neutropenia, in whom pyuria may be absent. [30] Recently, we showed that the most widely used pyuria cut-off (> 10 leukocytes/µl) has a low specificity for UTI in women ≥ 65 years, as asymptomatic bacteriuria is prevalent and is usually accompanied by intermediate degrees of pyuria. [31] As a cut-off of 200 leukocytes/µl increased the specificity to 86%, while maintaining a high sensitivity (89%), the core group incorporated these degrees of pyuria into the reference standard. An important modification to this domain after R2 was the removal of urine dipstick items (leukocyte esterase and nitrites) from the reference standard. Van den Broek et al. [32] show that leukocyte esterase results correlate poorly with absolute degrees of pyuria. Moreover, the core group believed that, at least in research studies, pyuria should be quantified to ensure the validity of the test results, improve comparability between studies and allow for better distinction from asymptomatic bacteriuria. However, quantification of pyuria may not be feasible in every research setting, such as primary and long-term care settings. Since UTI is frequently encountered in these healthcare settings and given the potential benefits of high-quality and standardised UTI research in primary and long-term care, the core group included a supplementary version of the reference standard, in which urine dipstick items are incorporated (see **Supplementary Figure 4**).

Culture results

During expert panel rounds, there was clear support for a threshold of 10^4 CFU/mL for 'significant' bacteriuria, which is lower than the threshold used in FDA and EMA guidelines.[8-10] The traditional threshold of 10^5 CFU/mL was also not supported in the aforementioned Delphi study on urine culture ordering, as it could lead to undertreatment of symptomatic patients with lower colony counts, and inappropriate treatment of asymptomatic patients with higher colony counts. [21] Moreover, the majority of current UTI studies included in our systematic review used thresholds below 10^5 CFU/mL. [11] Based on evidence supporting lower colony counts in symptomatic women with *Escherichia coli* bacteriuria, a threshold of 10^3 CFU/mL specifically for *Escherichia coli* was incorporated into the reference standard, as it is the causative pathogen in approximately 80% of cases. [28, 29, 33] In both systemic criteria and culture domains, points are awarded for bacteraemia (if pathogen matches urine culture results), as the core group felt that this finding represented the strongest evidence of UTI, and a maximum number

of points (5 points) should be given. Based on the study by Hooton et al. [28], enterococci and group B streptococci were not included in the typical uropathogen list (and their score was limited to 1 point). However, if enterococci and group B streptococci grow alongside a typical uropathogen, 3 points are still awarded for the typical uropathogen.

Strengths and limitations

Strengths of our study include using a well-described consensus methodology, the inclusion of experts from multiple relevant specialties and different countries, and requiring a high level of consensus (which was defined a priori). Decisions for the reference standard were not solely based on expert opinion, but also on best available evidence. Given that UTI diagnosis involves many factors, there is no single definitive test, and in clinical practice there are degrees of certainty when diagnosing UTI, we included a scoring system to reflect this, i.e. by including possible, probable, and definite UTI categories. There are several limitations to be noted. As a result of the multifaceted nature of UTI diagnosis, the reference standard does possess a certain level of complexity. However, accuracy was considered more important than simplicity, as the scoring system could be incorporated into a syntax, and this reference standard was not intended to be a clinical decision tool. Another limitation is that our reference standard does not apply to catheter-associated UTI. As symptom presentation and interpretation of urinalysis and culture results is even more challenging in this population, the core group believed that a separate reference standard should be developed for catheter-associated UTI studies. Moreover, a limitation of R1 specifically is that items were graded in isolation, while UTI diagnosis is usually based on many different factors, which might have influenced expert grading. Also, the expert panel consisted only of European and North American experts, and as such, the perspective of low-middle income countries is not represented. Finally, the question remains how a research reference standard can be validated in absence of an existing consensus-based reference standard for UTI. The partial validation that was carried out in our study by comparing case vignette interpretations to reference standard results could be repeated with a larger set of cases and blinded experts. [34] Ultimately, the true value of the reference standard will be determined by whether future UTI studies will adhere to the reference standard and whether this will lead to increased homogeneity between UTI studies.

In conclusion, we have established a consensus-based reference standard for UTI studies, which is supported by experts from multiple countries and medical specialties. This reference standard addresses a significant gap in UTI-related research and has the potential to improve both the internal and external validity of future UTI studies and facilitate accumulation of knowledge and evidence for a disease that imposes a substantial burden on individual patients and society as a whole.

Funding

No funding was received for this project.

Author contributions

Conceptualisation (M.P.B., S.P.C., C.S., T.N.P., C.N., L.M., J.M.C., S.E.G., B.K., F.W., M.K., M.M.C.L., L.G.V.), methodology (M.P.B., S.P.C., M.K., M.M.C.L.), data collection, curation and analysis (M.P.B.), writing – original draft preparation (M.P.B., M.M.C.L.), writing – review and editing (S.P.C., C.S., T.N.P., C.N., L.M., J.M.C., S.E.G., B.K., F.W., M.K., M.M.C.L., L.G.V.), supervision (S.P.C., M.M.C.L., L.G.V.). Two authors (M.P.B. and M.M.C.L.) have directly accessed and verified the underlying data reported in the manuscript. All authors have read and agreed to the final version of the manuscript.

Acknowledgements

The authors would like to acknowledge all experts participating in the survey rounds, listed under ‘UTI reference standard consensus group’. Furthermore, the authors would like to express their gratitude to Manon Zuurmond for her artistic contributions, which have greatly improved our figures.

Conflicts of interest

S.P.C. reports modest royalties for book editing roles for Oxford University Press and Springer. L.M. reports a grant from NIH - National Institute on Aging (PI on NIA funded T32 program, PI of a K24 mentorship award, Pepper Center Core Director for an NIA funded P30), a grant from Veterans Affairs (VA CSRD Merit Review Grant/Award to conduct research in Veteran population), a grant from CDC via Abt Associates (CDC has given a contract to Abt Associates to study viral transmission dynamics in nursing homes. U of Michigan has received a subcontract from Abt associates to study viral shedding among nursing home residents and staff affected by COVID-19), a grant from Betty and D. Dan Kahn Foundation (to study nasal microbiome in older nursing home patients), personal fees from NIH (to serve on study sections), personal fees from Up-to-date (to serve as an author and editor for its 'Infection and Aging' section), personal fees from University of Connecticut OAIC Pepper Center External Advisory Board (to serve on the external advisory board of an NIA funded P30), and personal fees from Northwestern University OAIC Pepper Center (to serve on the external

advisory board of an NIA funded P30). J.M.C. was supported by a grant from the National Institutes of Health, National Institute on Aging (R01 AG050801). S.E.G. reports receiving consulting fees for being on the international advisory board of Immunotek regarding the MV140 vaccine, and being a consultant for Biomerieux regarding the development of diagnostics tests (fees paid to institution). F.W. reports receiving consulting fees for being an advisor/consultant for Venatorx Pharmaceuticals, Glaxo Smith Kline, Spero Pharmaceuticals, and Bionorica. F.W. was supported by Bionorica and Glaxo Smith Kline for attending meetings and/or travel. F.W. has received honoraria for speaking at events for Astellas, AstraZeneca, Bionorica, Glaxo Smith Kline, Janssen, Klosterfrau, MIP Pharma, OM Pharma, and Pfizer. F.W. has been part of an advisory board of Achaogen, AstraZeneca, Bionorica, Janssen, LeoPharma, MerLion, MSD, OM Pharma/Vifor Pharma, Pfizer, RosenPharma, Shionogi, Venatorx Pharmaceuticals, and Glaxo Smith Kline. F.W. has contributed to the development of the German S3 guideline for urinary tract infections, and the Infections in Urology guideline of the European Association of Urology. F.W. reports prior study participation for Achaogen, Bionorica, Enteris BioPharma, Helperby Therapeutics, OM Pharma/Vifor Pharma, Shionogi, Deutsches Zentrum für Infektionsforschung, and being a speaker for the German Research Foundation (Bacterial Renal Infections and Defense). M.M.C.L. reports having received a grant for being a Principal Investigator of the Embrace study. L.G.V. reports being a co-investigator in a stage III pharmacy-driven vaccine trial to prevent septicaemia for UTI by Janssen. All other authors (M.P.B., C.S., T.N.P., C.N., B.K., M.K.) do not have any conflicts of interest. None of the funding agencies mentioned above had any role in the conduct of the work or writing of the manuscript.

Disclaimer

The contents presented herein do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Members of the UTI reference standard consensus group (expert panel)

- Thomas Hooton (Department of Medicine, University of Miami, USA)
- Lindsay Nicole (University of Manitoba, Winnipeg, Canada)
- Barbara Trautner (Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, USA)
- Kalpana Gupta (Boston Veterans Affairs Healthcare System and Boston University School of Medicine, USA)
- Dimitri Drekonja (Department of Medicine, University of Minnesota, USA)
- Angela Huttner (Infectious Diseases Division, Geneva University Hospitals and School of Medicine, Switzerland)
- Laila Schneidewind (Department of Urology, University Medical Centre Rostock, Germany)
- Truls Erik Bjerklund Johansen (Department of Urology, Oslo University Hospital, Norway)
- José Medina-Polo (Department of Urology, University Hospital 12 de Octubre, Spain)
- Jennifer Kranz (Department of Urology and Paediatric Urology, University Medical Center RWTH Aachen, Aachen, Germany; Department of Urology and Kidney Transplantation, Martin Luther University, Halle (Saale), Germany)
- Thijs ten Doesschate (Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands)
- Alewijn Ott (Department of Medical Microbiology, Certe, Groningen, The Netherlands)
- Sacha Kuil (Department of Infectious Diseases, Public Health Service of Amsterdam, Department of Medical Microbiology and Infection Prevention, University of Amsterdam, The Netherlands)
- Michael Pulia (BerbeeWalsh Department of Emergency Medicine, University of Wisconsin-Madison School of Medicine and Public Health, USA)
- Christopher Carpenter (Department of Emergency Medicine, Mayo Clinic-Rochester, USA)
- Janneke Stalenhoef (Department of Internal Medicine, OVLG Amsterdam, The Netherlands)
- Sophie Clark (Geriatrics, University of Colorado, USA)
- Lauren Southerland (Department of Emergency Medicine, The Ohio State University Wexner Medical Center, USA)
- Brynjar Fure (School of Medical Sciences, Örebro University, Sweden)

- Evert Baten (Department of Urology, University Hospitals Leuven, Belgium)
- Sean Ninan (Leeds and York Partnership NHS Foundation Trust, UK)
- Lara Gerbrandy-Schreuders (Department of Urology, Amsterdam UMC, The Netherlands)
- Karlijn van Halem (Department of Infectious Diseases and Immunity, Jessa Hospital, Belgium)
- Marco Blanker (Department of Primary and Long-term Care, University Medical Center Groningen, The Netherlands)
- Kurt Naber (Department of Urology, Technical University of Munich, Germany)
- Adrian Pilatz (Clinic for Urology, Pediatric Urology and Andrology, Justus-Liebig University, Germany)
- Stefan Heytens (Department of Public Health and Primary Care, Faculty of Medicine and Health Sciences, Ghent University, Belgium)
- Ali Vahedi (School of Medical Sciences, Örebro University, Sweden)
- David Talan (David Geffen School of Medicine, UCLA, Los Angeles, California, USA)
- Ed Kuijper (Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, The Netherlands)
- Jaap van Dissel (Department of Infectious Diseases, Leiden University Medical Center, The Netherlands)
- Jochen Cals (School for Public Health and Primary Care, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands)
- Sarah Dubbs (Department of Emergency Medicine, University of Maryland School of Medicine, USA)
- Rajan Veeratterapillay (Freeman Hospital, Newcastle Upon Tyne, UK)
- Pär-Daniel Sundvall (General Practice/Family Medicine, School of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden)
- Silvia Bertagnolio (University College London, UK)
- Christopher Graber (Infectious Diseases Section, Department of Medicine, VA Greater Los Angeles Healthcare System, Los Angeles, California, USA)
- Wouter Rozemeijer (Department of Medical Microbiology, Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands)
- Robin Jump (Geriatric Research Education and Clinical Center (GRECC) at the VA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, USA)
- Ildiko Gagyor (Department of General Practice, University Hospital Würzburg, Germany)

- Ingvild Vik (The Antibiotic Centre for Primary Care, Department of General Practice, Institute of Health and Society, University of Oslo, Norway)
- Karola Waar (Izore, Centre for Infectious Diseases Friesland, The Netherlands)
- Martha van der Beek (Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, The Netherlands)

References

1. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am **2014**; 28(1): 1-13.
2. Ennis SS, Guo H, Raman L, Tambyah PA, Chen SL, Tiong HY. Premenopausal women with recurrent urinary tract infections have lower quality of life. Int J Urol **2018**; 25(7): 684-9.
3. Davenport M, Mach KE, Shortliffe LMD, Banaei N, Wang TH, Liao JC. New and developing diagnostic technologies for urinary tract infections. Nat Rev Urol **2017**; 14(5): 296-310.
4. Sihra N, Goodman A, Zakri R, Sahai A, Malde S. Nonantibiotic prevention and management of recurrent urinary tract infection. Nat Rev Urol **2018**; 15(12): 750-76.
5. de Groot JA, Bossuyt PM, Reitsma JB, et al. Verification problems in diagnostic accuracy studies: consequences and solutions. BMJ **2011**; 343: d4770.
6. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) Events. Available at: https://www.cdc.gov/nhsn/psc/uti/. Accessed 8th of June.
7. Stone ND, Ashraf MS, Calder J, et al. Surveillance definitions of infections in long-term care facilities: revisiting the McGeer criteria. Infect Control Hosp Epidemiol **2012**; 33(10): 965-77.
8. Evaluation of medicinal products indicated for treatment of bacterial infections. Available at: https://www.ema.europa.eu/en/evaluation-medicinal-products-indicated-treatment-bacterial-infections Accessed 8th of June.
9. Uncomplicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/uncomplicated-urinary-tract-infections-developing-drugs-treatment-guidance-industry Accessed 8th of June.
10. Complicated Urinary Tract Infections: Developing Drugs for Treatment. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/complicated-urinary-tract-infections-developing-drugs-treatment Accessed 8th of June.
11. Bilsen MP, Jongeneel RMH, Schneeberger C, et al. Definitions of Urinary Tract Infection in Current Research: A Systematic Review. Open Forum Infect Dis **2023**; 10(7): ofad332.
12. Junger S, Payne SA, Brine J, Radbruch L, Brearley SG. Guidance on Conducting and REporting DElphi Studies (CREDES) in palliative care: Recommendations based on a methodological systematic review. Palliat Med **2017**; 31(8): 684-706.
13. Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. J Clin Epidemiol **2014**; 67(4): 401-9.
14. McMillan SS, King M, Tully MP. How to use the nominal group and Delphi techniques. Int J Clin Pharm **2016**; 38(3): 655-62.
15. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform **2019**; 95: 103208.

16. Hsu C. C. SBA. The Delphi Technique: Making Sense of Consensus. Practical Assesment, Research, and Evaluation **2007**; 12(10).

17. van Buul LW, Vreeken HL, Bradley SF, et al. The Development of a Decision Tool for the Empiric Treatment of Suspected Urinary Tract Infection in Frail Older Adults: A Delphi Consensus Procedure. J Am Med Dir Assoc **2018**; 19(9): 757-64.

18. Fitch K, Bernstein S, M. A. The RAND/UCLA Appropriateness Method User's Manual. Santa Monica: RAND Corporation. , **2001**.

19. Bent S, Nallamothu BK, Simel DL, Fihn SD, Saint S. Does this woman have an acute uncomplicated urinary tract infection? JAMA **2002**; 287(20): 2701-10.

20. Gupta K, Hooton TM, Roberts PL, Stamm WE. Patient-initiated treatment of uncomplicated recurrent urinary tract infections in young women. Ann Intern Med **2001**; 135(1): 9-16.

21. Claeys KC, Trautner BW, Leekha S, et al. Optimal Urine Culture Diagnostic Stewardship Practice-Results from an Expert Modified-Delphi Procedure. Clin Infect Dis **2022**; 75(3): 382-9.

22. Nicolle LE, Gupta K, Bradley SF, et al. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis **2019**; 68(10): 1611-5.

23. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis **2008**; 40(10): 804-10.

24. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc **1995**; 43(7): 772-5.

25. van Nieuwkoop C, Bonten TN, van't Wout JW, et al. Procalcitonin reflects bacteremia and bacterial load in urosepsis syndrome: a prospective observational study. Crit Care **2010**; 14(6): R206.

26. Lawandi A, Oshiro M, Warner S, et al. Reliability of Admission Procalcitonin Testing for Capturing Bacteremia Across the Sepsis Spectrum: Real-World Utilization and Performance Characteristics, 65 U.S. Hospitals, 2008-2017. Crit Care Med **2023**.

27. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med **2013**; 41(2): 580-637.

28. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med **2013**; 369(20): 1883-91.

29. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med **1982**; 307(8): 463-8.

30. Klaassen IL, de Haas V, van Wijk JA, Kaspers GJ, Bijlsma M, Bokenkamp A. Pyuria is absent during urinary tract infections in neutropenic patients. Pediatr Blood Cancer **2011**; 56(5): 868-70.

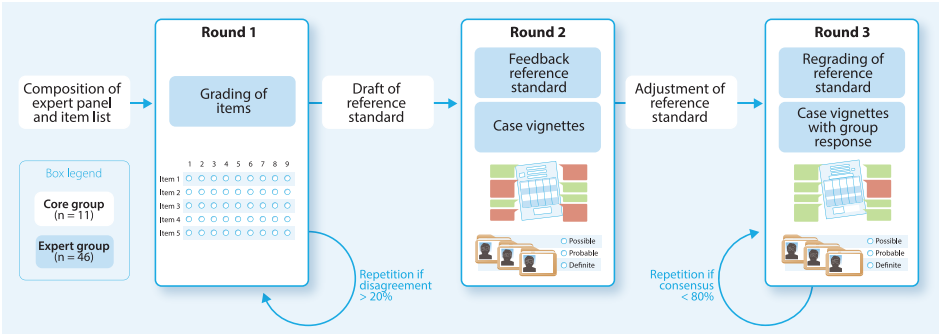
31. Bilsen MP, Aantjes MJ, van Andel E, et al. Current Pyuria Cutoffs Promote Inappropriate Urinary Tract Infection Diagnosis in Older Women. Clin Infect Dis **2023**; 76(12): 2070-6.

32. van den Broek D, Keularts IM, Wielders JP, Kraaijenhagen RJ. Benefits of the iQ200 automated urine microscopy analyser in routine urinalysis. Clin Chem Lab Med **2008**; 46(11): 1635-40.

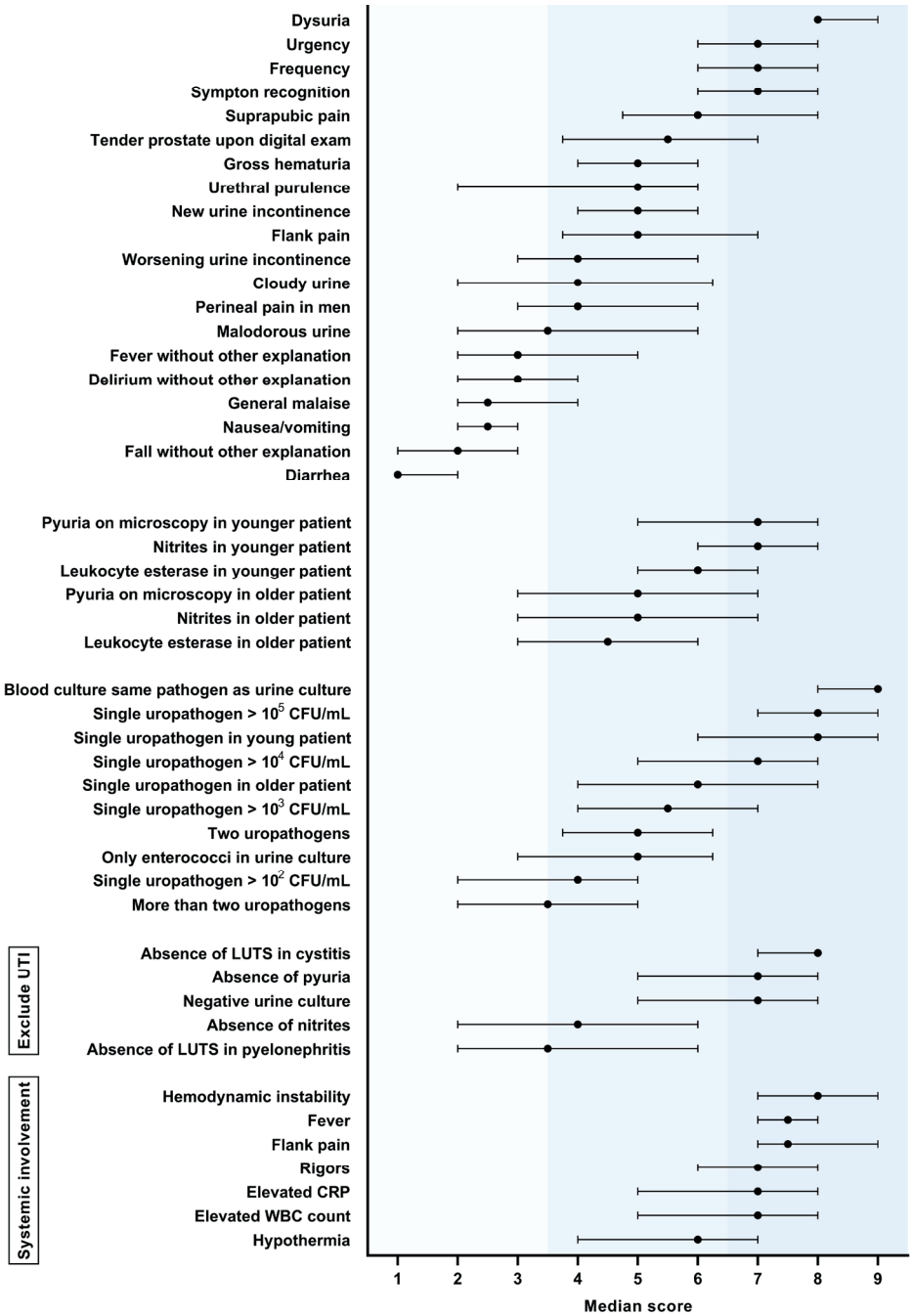
33. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol **2015**; 13(5): 269-84.

34. Moore CL, Carpenter CR, Heilbrun ME, et al. Imaging in Suspected Renal Colic: Systematic Review of the Literature and Multispecialty Consensus. Ann Emerg Med **2019**; 74(3): 391-9.

Supplement

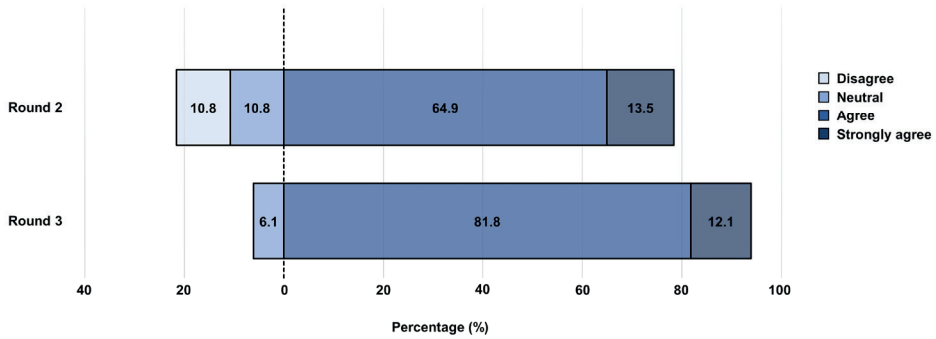


Supplementary Figure 1: Overview of the study design. The core group prepared a questionnaire for the expert group comprising 48 items related to urinary tract infection (UTI) diagnosis. In round 1, the expert panel assigned a value to each item on a Likert scale, ranging from 1 ('not at all indicative of UTI') to 9 ('highly indicative of UTI'). If disagreement (definition according to RAND/UCLA Appropriateness Method) occurred in more than 20% of the items, we planned to conduct another round. Based on the results of round 1 and the available evidence, the core group developed a reference standard. In round 2, consensus was assessed in two ways: experts were asked to rate a set of case vignettes (to evaluate alignment with the reference standard) and provide direct feedback on the initial version of the reference standard. In round 3, experts re-evaluated the same case vignettes and the revised reference standard. If consensus (defined as a minimum of 80% of experts voting 'agree' or 'strongly agree' and none of the experts voting 'disagree') was not reached after round 3, further rounds were planned until consensus was achieved.

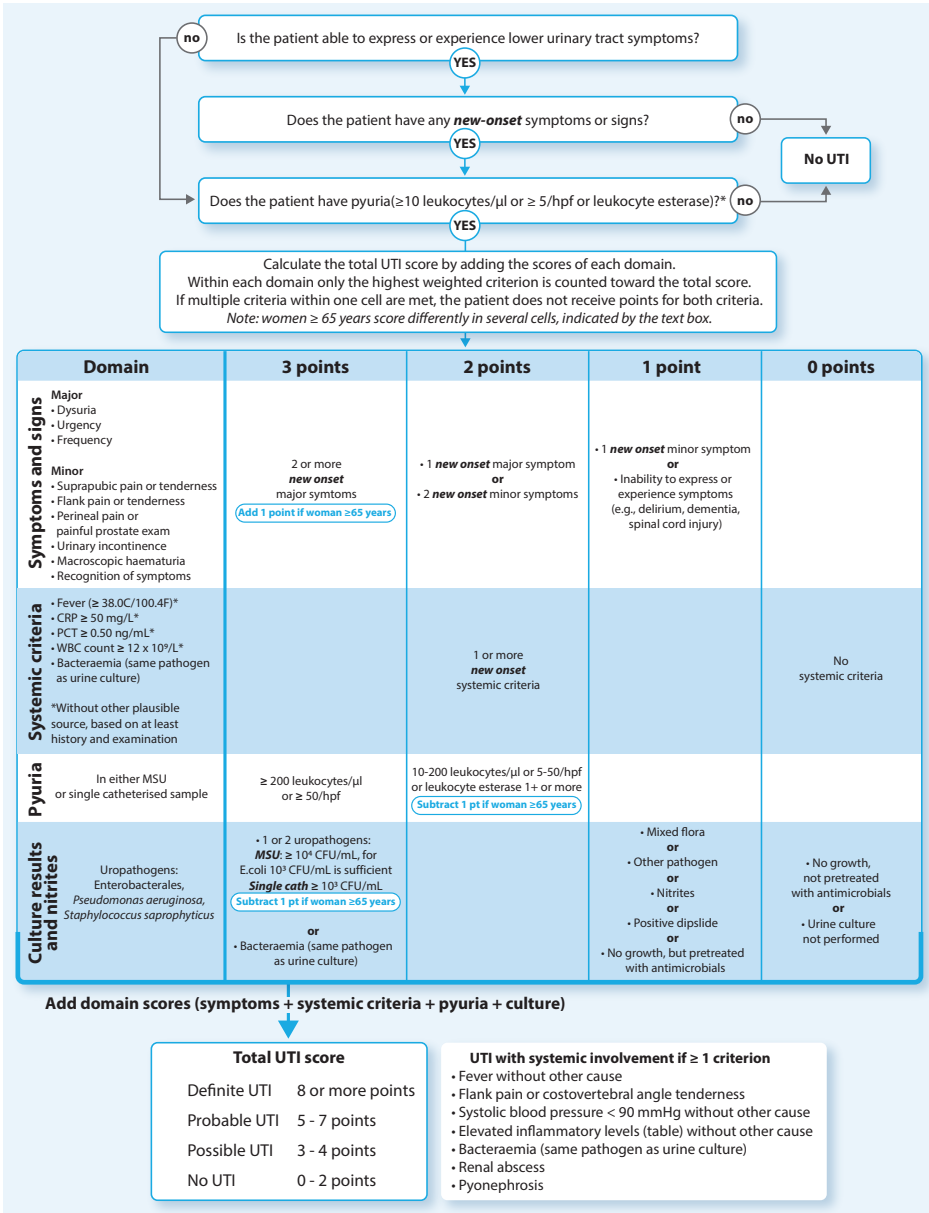


Supplementary Figure 2: Delphi round 1 results. Per item, median scores (represented by the dot) and interquartile ranges are shown. An item was deemed indicative of urinary tract infection (UTI) in case of a panel median ≥ 6·5 (blue panel) without disagreement, not indicative of UTI in case

of a panel median of ≤ 3·5 (white panel) without disagreement, and uncertain if the panel median lay in between indicative and not indicative (light blue panel), or any median with disagreement. Disagreement (both extremes of the Likert scale containing more than a third of responses) did not occur. For the 'exclude UTI' items, a high median score indicates that the item rules out UTI. Abbreviations: CFU = colony-forming units, LUTS = lower urinary tract symptoms; CRP = C-reactive protein; WBC = white blood cell



Supplementary Figure 3: Likert plot of reference standard consensus in Delphi rounds 2 and 3. The 5-point Likert scale ranged from 'strongly disagree' to 'strongly agree'. None of the experts voted for 'strongly disagree'. Experts who neither disagreed nor agreed with the reference standard are depicted as 'neutral'. The proportion of experts voting for each Likert option is displayed in the corresponding bar. Consensus was defined a priori as a minimum of 80% of experts voting 'agree' or 'strongly agree' and none of the experts voting 'disagree'



Supplementary Figure 4: Research reference standard for urinary tract infections – supplement. * In case of obstructive uropathy or absolute neutropenia, pyuria may be absent and the total UTI score may be calculated. Of note: obtaining a urine sample is of utmost importance in all study populations and settings. If no urine can be obtained (neither midstream nor through single catheterization) the total UTI score may be calculated, but this should be mentioned in your study limitations. Abbreviations: UTI = urinary tract infection, CRP = C-reactive protein, PCT = procalcitonin, WBC = white blood cell, MSU = midstream urine, CFU = colony-forming units.

Supplementary Table 1: Concordance between case vignette results and reference standard.

Case (abbreviated)	Ref. standard	Round	Definite	Probable	Possible	No UTI
F25, at GP, new-onset dysuria and frequency, no fever, dipstick positive for LE and nitrites, no urine culture performed.	Probable	2	19 (48)	20 (50)	1 (3)	0
		3	16 (43)	21 (57)	0	0
F80, at LTCF, ADL dependent, refuses morning care because 'she just does not feel like it', no history of cognitive impairment, no signs of delirium, no flank pain, no LUTS, no fever, dipstick positive for LE and nitrites, urine culture *E. coli* > 10^5 CFU/mL.	No UTI	2	3 (8)	7 (18)	12 (30)	18 (45)
		3	1 (3)	3 (8)	10 (27)	23 (62)
M70, at ED, history of BPH, new-onset urgency and frequency, fever, CRP 150 mg/L, urine microscopy 800 leukocytes/µl (> 50 leukocytes/hpf), urine and blood culture *K. pneumoniae* > 10^5 CFU/mL.	Definite	2	37 (93)	2 (5)	1 (3)	0
		3	37 (100)	0	0	0
M85, at home, history of MCI, signs of delirium over the last day, incoherent answers when questioned about LUTS, fever present, no apparent source of infection upon examination, no urine sample due to aggression	Possible	2	0	5 (13)	34 (85)	1 (3)
		3	1 (3)	1 (3)	35 (95)	0
F70, at outpatient clinic, new-onset urinary incontinence and urgency, no other LUTS, no flank pain, no fever, urine microscopy no leukocytes, urine culture mixed flora.	No UTI	2	0	1 (3)	11 (28)	28 (70)
		3	0	1 (3)	6 (16)	30 (81)
F20, at ED, new-onset flank pain and dysuria, no other LUTS, fever is present, CRP 100 mg/L, urine microscopy 500 leukocytes/µl, urine culture *E. coli* > 10^4 CFU/mL, blood culture no growth.	Definite	2	31 (78)	6 (15)	2 (5)	1 (3)
		3	35 (95)	1 (3)	1 (3)	0
F75, at GP, new-onset frequency, no other LUTS, no flank pain, no fever, urine dipstick positive for LE, no nitrites, urine culture *E. faecalis* > 10^4 CFU/mL.	Possible	2	5 (13)	9 (23)	19 (48)	7 (18)
		3	3 (8)	3 (8)	25 (68)	6 (16)

Supplementary Table 1: Continued

Case (abbreviated)	Ref. standard	Round	Definite	Probable	Possible	No UTI
F45, calls GP, dysuria and suprapubic pain, started one day prior, no other LUTS, no fever, no flank pain, took one dose of oral fosfomycin a day ago as patient recognised symptoms, urine dipstick positive for LE, no nitrites, urine culture no growth.	Probable	2	6 (15)	28 (70)	5 (13)	1 (3)
		3	1 (3)	31 (84)	4 (11)	1 (3)
F85, at outpatient clinic, new-onset gross haematuria, oral anticoagulant use, no other LUTS, no flank pain, no fever, urine microscopy 50 leukocytes/µl and 1500 erythrocytes/µl, urine culture *E. coli* and *P. aeruginosa* both > 10^4 CFU/mL.	Possible	2	2 (5)	5 (13)	17 (43)	16 (40)
		3	0	0	22 (60)	15 (41)
F75, at GP, new-onset dysuria, frequency and urgency, no flank pain, no fever, urine dipstick positive for LE and nitrites, urine culture shows mixed flora.	Probable	2	5 (13)	22 (55)	12 (30)	1 (3)
		3	3 (8)	25 (68)	9 (24)	0

All values are n (%). In round 2, 40 experts answered all case vignettes, blinded to the reference standard and group results. In round 3, 37/40 experts (93%) regraded the same case vignettes after having seen group results of round 2. To evaluate alignment between the reference standard and case vignettes in which urine dipsticks were used, we applied the supplementary reference standard. Abbreviations: UTI = urinary tract infection, F = female, M = male, GP = general practitioner, LE = leukocyte esterase, LTCF = long-term care facility, ADL = activities of daily living, LUTS = lower urinary tract symptoms, CFU = colony-forming units, ED = emergency department, BPH = benign prostatic hyperplasia, CRP = C-reactive protein, MCI = mild cognitive impairment.

Supplementary Table 2: Delphi round 2 expert panel comments.

Case number	Expert panel comments
1	• Urine culture result required for definite diagnosis (n = 6) • Could also be sexually transmitted infection or Candidiasis (n = 5)
2	• This is a clear case of asymptomatic bacteriuria (n = 9) • I would wait and see how symptoms develop (n = 3) • Non-specific symptoms are indicative of UTI (n = 3) • Further testing is required/other infections should be ruled out (n = 2)
3	• No remarkable comments
4	• Evaluation of other causes is necessary/source unclear (n = 7) • Delirium and fever are likely UTI (n = 6)
5	• No UTI because of absence of pyuria (n = 3) • New-onset symptoms could be UTI (n = 3) • Would repeat urine culture (n = 3)
6	• Likely pyelonephritis (n = 5) • Further imaging is needed, renal stone (n = 2) • Symptoms more important than bacterial count (n = 2)
7	• Could also be overactive bladder/rule out other cause (n = 5) • Enterococci can be uropathogens (n = 2) • Sample quality (epithelial cells) should be provided (n = 1)
8	• Urine culture probably negative due to pretreatment (n = 10) • Symptom recognition is most important here (n = 2)
9	• Could be bladder cancer/stones, needs cystoscopy (n = 9) • Probably ASB (n = 3) • Would treat because of haematuria (n = 1)
10	• Contaminated specimen, new culture needed (n = 6)

Abbreviations: UTI = urinary tract infection, ASB = asymptomatic bacteriuria

Part II

Diagnostic challenges

Chapter 4

Current pyuria cut-offs promote inappropriate urinary tract infection diagnosis in older women

Manu P. Bilsen, Margaretha J. Aantjes, Esther van Andel, Janneke E. Stalenhoef, Cees van Nieuwkoop, Eliane M.S. Leyten, Nathalie M. Delfos, Martijn Sijbom, Mattijs E Numans, Wilco P. Achterberg, Simon P. Mooijaart, Martha T. van der Beek, Christa M. Cobbaert, Simon P. Conroy, Leo G. Visser, Merel M.C. Lambregts

Clin Infect Dis. 2023 Jun 16;76(12):2070-2076

Abstract

Background

Pre-existing lower urinary tract symptoms (LUTS), cognitive impairment and the high prevalence of asymptomatic bacteriuria (ASB) complicate the diagnosis of urinary tract infection (UTI) in older women. The presence of pyuria remains the cornerstone of UTI diagnosis. However, over 90% of ASB patients have pyuria, prompting unnecessary treatment. We quantified pyuria by automated microscopy and flowcytometry to determine the diagnostic accuracy for UTI and to derive pyuria thresholds for UTI in older women.

Methods

Women ≥ 65 years with ≥ 2 new-onset LUTS and one uropathogen ≥ 10^4 colony-forming units/mL (CFU/mL) were included in the UTI-group. Controls were asymptomatic and classified as ASB (one uropathogen ≥ 10^5 CFU/mL), negative culture or mixed flora. Patients with an indwelling catheter or antimicrobial pretreatment were excluded. Leukocyte medians were compared and sensitivity-specificity pairs were derived from a receiver operating characteristic-curve.

Results

We included 164 participants. UTI patients had higher median urinary leukocytes compared to control patients (microscopy: 900 versus 26 leukocytes/µl; flowcytometry 1575 versus 23 leukocytes/µl, $p < 0.001$). Area under the curve was 0.93 for both methods. At a cut-off of 264 leukocytes/µl, sensitivity and specificity of microscopy were 88% (positive and negative likelihood ratio 7.2 and 0.1, respectively). The commonly used cut-off of 10 leukocytes/µl had a poor specificity (36%) and a sensitivity of 100%.

Conclusion

The degree of pyuria can help to distinguish UTI in older women from ASB and asymptomatic controls with pyuria. Current pyuria cut-offs are too low and promote inappropriate UTI diagnosis in older women.

Introduction

Urinary tract infection (UTI) incidence increases with age and is higher in women than in men. [1] In older women, diagnosing UTI is complicated for several reasons. Firstly, symptom communication may be affected by cognitive impairment. Secondly, pre-existing lower urinary tract symptoms (LUTS), such as urinary incontinence and urgency, are common and distinguishing acute from chronic LUTS can be challenging. [2] Finally, 20% of community-dwelling and 50% of institutionalised older women have asymptomatic bacteriuria (ASB), defined as the presence of one or more uropathogens ≥ 10^5 colony-forming units per millilitre (CFU/mL) in the absence of signs or symptoms attributable to UTI. [3-5] As a result, inappropriate antimicrobial treatment is common, leading to unnecessary side effects, drug interactions, *Clostridioides difficile* infection and the selection of antimicrobial resistant pathogens. [6, 7] Distinguishing ASB from UTI is further complicated by the fact that over 90% of older women with ASB have concomitant pyuria. [8, 9] Consequently, the positive predictive value of the presence of pyuria for UTI is low in older women. However, it is unclear whether the degree of pyuria differs between older women with UTI and ASB, partly because urine dipstick is the most ordered screening test, providing only semi-quantitative results of leukocyte esterase activity. Pyuria can be quantified in different ways. Initially, Mabeck et al. [10] found that a leukocyte excretion rate of 400,000 per hour could distinguish UTI from asymptomatic women. This rate corresponds with a cut-off value of 10 leukocytes/mm^3 in unspun urine. [11] In clinical practice and research, pyuria is most often quantified by direct or automated microscopy of (un)spun urine, usually after initial dipstick screening. Automated microscopy reduces variability in centrifugation and resuspension of urine and is more efficient than direct microscopy. [12] In recent years, an increasing amount of laboratories are adopting urine flowcytometry for quantification of pyuria. Although cut-off values for 'significant' pyuria vary in the literature and depend on quantification methods, commonly accepted cut-offs include 10 leukocytes/µl and 5-10 leukocytes per high-powered field (hpf). These cut-off values are largely derived from studies involving non-pregnant premenopausal women, in whom ASB is uncommon. [13] The objective of this study was to determine sensitivity and specificity of automated microscopy and urine flowcytometry for diagnosing UTI in older women, with the ultimate goal to derive optimal cut-off values for pyuria for UTI in this population, taking ASB into account.

Methods

This study is an exploratory analysis of an overarching, case-control study registered at the International Clinical Trials Registry Platform (NL9477). The study was conducted across five hospitals (four regional and one academic), four long-term care facilities (LTCF), three primary care centres, one after-hours primary care clinic, and fourteen senior housing facilities. This study was approved by the regional medical ethics committee (METC-LDD) and was conducted in accordance with the declaration of Helsinki. [14] Written informed consent was obtained from all participants.

Study population

Women aged ≥ 65 years were eligible for inclusion. Exclusion criteria included inability to express symptoms (e.g. due to delirium or cognitive impairment), the presence of an indwelling catheter, immunosuppressive use, antimicrobial use (< 48 hours prior to inclusion), current urolithiasis, and a UTI in the previous month. Stringent criteria were applied to both UTI and control patients, as a consensus-based reference standard for UTI is currently missing. To be eligible for the UTI group, patients were required to have at least two new-onset LUTS (dysuria, frequency, urgency, or suprapubic pain). Furthermore, patients were required to have pyuria, defined as ≥ 10 leukocytes/µl or ≥ 5 leukocytes/hpf or presence of leukocyte esterase, and a monoculture, i.e. one uropathogen ≥ 10^4 CFU/mL for the primary analysis. Enterobacterales, enterococci, *Pseudomonas aeruginosa, Staphylococcus saprophyticus,* and group B streptococci were considered uropathogens. In case of a temperature ≥ 38.0 °C, patients were classified as having an upper UTI. Community-dwelling women and LTCF residents who did not have any LUTS or fever were eligible as controls. Patients were eligible regardless of urine culture results and they were subdivided into three subgroups: ASB, negative culture and mixed flora. ASB was defined as at least two consecutive urine cultures (2 – 4 weeks apart) with the same uropathogen ≥ 10^5 CFU/mL, and a negative culture was defined as no growth or growth of non-pathogenic micro-organisms ≤ 10^3 CFU/mL. Cases and controls were not matched for age or comorbidities.

Study procedures and methods of measurement

The study team was contacted by the treating physician in case of a potential participant at the emergency department, LTCF or primary care office. Asymptomatic LTCF residents were asked to participate by their elderly-care

physician; community-dwelling older women were recruited through flyers. If eligibility criteria were met, participants were visited by the study team within 1 hour. Baseline data included: age, prior medical history (hypertension, chronic kidney disease, diabetes mellitus and urological history), new-onset LUTS and fever. All patients underwent a delirium screening and assessment of dependency in activities of daily living (ADL) through 4AT and Katz questionnaires respectively, and measurement of vital signs. [15, 16]

Urinalysis

Midstream urine was collected in a 100 mL sterile urine container. Urine obtained via single catheterisation was accepted, urine collected from a bedpan was not. After collection, the urine was divided into two V-monovette 10 mL urine tubes (Sarstedt, Nümbrecht, Germany), one for automated microscopy and one for urine flowcytometry. Automated microscopy was performed using the Cobas U701 (Roche, Rotkreuz, Switzerland). [17] After mixing by the analyser, 170 µl of urine was injected into a polycarbonate cuvette. Next, a monolayer of cells was created by centrifuging the cuvette for 10 seconds at 260 g. Cobas U701 output included quantitative measures of leukocytes in cells/µl with a lower limit of detection (LLD) of 1 cell/µl and an upper limit of detection (ULD) of 900 cells/µl. Urine flowcytometry was carried out with the Sysmex UF-4000 (Sysmex, Kobe, Japan). Within the analyser, fluorescent dyes were added to 450 µl of urine, after which urine particles were quantified and classified by analysis of scattered light patterns. LLD was 1 leukocyte/µl and ULD was 10,000 leukocytes/µl. All urine samples were analysed in the Leiden University Medical Center, except for urine samples of the participants who were included in regional hospitals. In the latter case, urine was analysed in the corresponding regional hospital by automated microscopy, as urine flowcytometry was not available. All urine samples were kept at room temperature and analysed within 4 hours of micturition to ensure stability of all urine components.

Microbiological assessments

The remaining urine in the sterile container was used for bacteriological culture at the microbiology department. For all included UTI and control patients, 10 µL of non-centrifuged urine was placed on routine culture media and incubated for one day. A culture result was deemed positive in case of growth ≥ 10^4 CFU/

mL and defined as a monoculture if ≥ 90% of the cultured colonies were of one micro-organism.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM, Armonk, USA). Data are presented as percentages, means with standard deviations, or medians with interquartile ranges as appropriate. A Mann-Whitney U test was performed to compare leukocyte medians between UTI patients and controls. As a pyuria threshold for UTI in older women is not known, sensitivity-specificity pairs with associated 95% confidence intervals (95% CI) were calculated for all possible cut-offs and plotted in a receiver operating characteristic (ROC) curve using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California USA). The area under the curve (AUC) was calculated to determine the discriminative ability of the index tests (automated microscopy and urine flowcytometry). Youden's index was used to determine the cut-off value with the optimal trade-off between sensitivity and specificity. In a fraction of UTI cases automated microscopy results were missing, e.g. only semi-quantitative results were available (leukocyte esterase or leukocytes/hpf). The impact of missing automated microscopy results on estimates of accuracy was evaluated by a sensitivity analysis consisting of best- and worst-case scenarios (all missing pyuria results were either considered true positive and negative or false positive and negative respectively). Twenty-seven patients presenting with LUTS were not included in the primary analysis because they did not meet the urine culture criteria for the UTI group. Their urine leukocyte counts were evaluated separately, in the secondary analysis.

Results

Of the 213 screened participants, 199 were eligible for inclusion, of which 164 were included in the primary analysis (**Figure 1**). Baseline characteristics are summarised in **Table 1**. UTI and control groups were comparable in terms of age (overall mean 78.3 years) and comorbidities. Inclusion sites differed between UTI and control groups, e.g. 11% of UTI patients versus 43% of controls were included in a LTCF. ADL dependency scores were comparable. Within the UTI group, the most common new-onset symptom was frequency, followed by urgency and dysuria; 13/63 patients (21%) had an upper UTI.

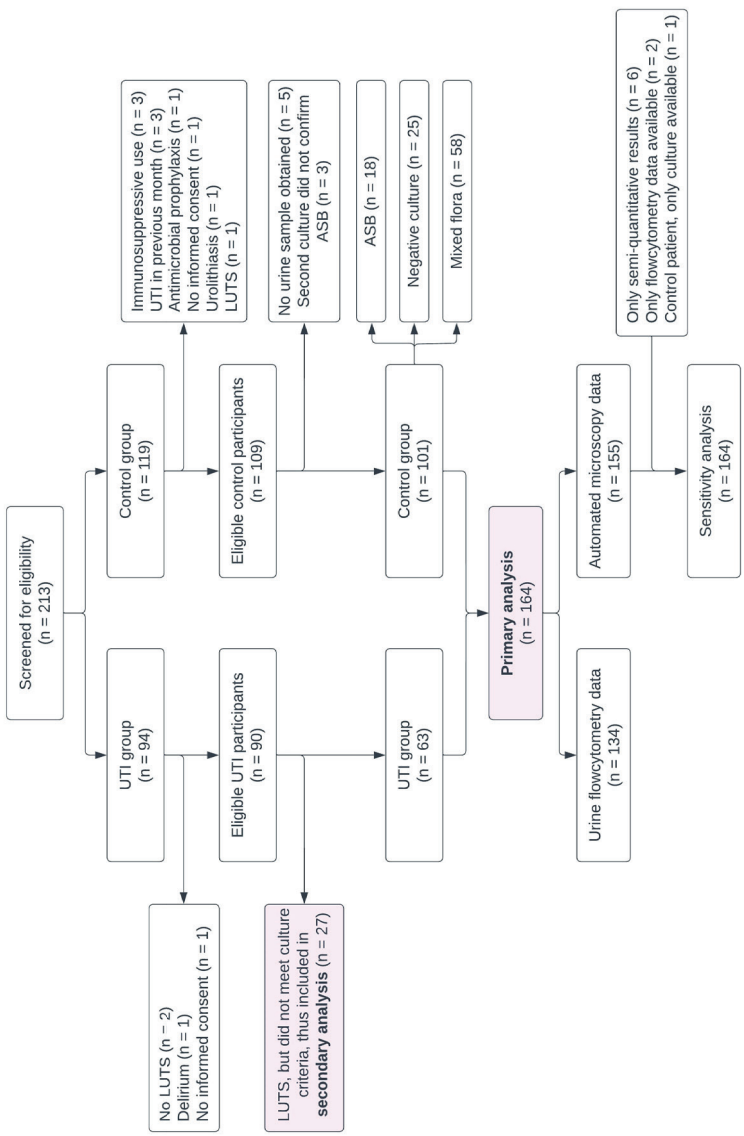


Figure 1: Overview of screening and selection process. All patients in the control group were asymptomatic. Abbreviations: UTI = urinary tract infection, LUTS = lower urinary tract symptoms, ASB = asymptomatic bacteriuria

Table 1: Baseline characteristics of UTI patients and controls.

Baseline characteristics	UTI (n = 63)	Controls (n = 101)
Age in years	77.1 (8.0)	79.0 (8.0)
Setting		
Hospital	18 (28.6)	0
LTCF	7 (11.1)	43 (42.6)
Primary care office	38 (60.3)	0
At home	0	58 (57.4)
Urological history		
Cystocele/rectocele	3 (4.7)	3 (3.0)
Previous urolithiasis	2 (3.2)	1 (1.0)
Previous kidney/bladder malignancy	1 (1.6)	1 (1.0)
Urinary incontinence procedure	1 (1.6)	2 (2.0)
Bladder sphincterotomy	0	1 (1.0)
Other comorbidity		
Diabetes mellitus	14 (22.2)	14 (13.9)
Hypertension	30 (47.6)	47 (46.5)
History of CKD	12 (19.0)	11 (10.9)
UTI history		
Ever had UTI	57 (90.5)	77 (76.2)
Ever hospitalised for UTI	2 (3.2)	1 (1.0)
Number of UTI in past year	1 (0 – 2)	0 (0 – 0)
Antibiotics in previous month	16 (25.4)	20 (19.8)
New-onset symptoms	63 (100)	0
Dysuria	49 (77.8)	-
Frequency	57 (90.5)	-
Urgency	53 (84.1)	-
Suprapubic pain	43 (68.3)	-
Urethral pain	33 (52.4)	-
Flank pain	12 (19.0)	-
New/worsening urinary incontinence	31 (49.2)	-
Recognition of symptoms	46 (73.0)	-
Fever (≥ 38.0)	13 (20.6)	-
ADL-dependency ≥ 2 Katz-items	14 (22.2)	23 (23.8)

Age is expressed as mean (SD), number of UTI in past year as median (IQR), and all other variables are expressed as n (%). The living situation of hospitalised UTI patients was unknown. History of CKD was self-reported. One UTI patient had had renal cell carcinoma twelve years prior, and one control patient had had non-muscle-invasive bladder cancer two years prior. In both patients, there was no evidence of active malignancy. Fever was objectified, 13 patients had an upper UTI. Abbreviations: UTI = urinary tract infection, LTCF = long-term care facility, CKD = chronic kidney disease, ADL = activities of daily living

Nearly all urine samples were midstream samples (162/164 (98.8%)). ASB prevalence in our control group was 18%. Within the UTI group, *E. coli* was the most common causative pathogen (81%), followed by *Klebsiella* spp. (4.8%), and *Proteus mirabilis* (4.8%). Two episodes were caused by extended-spectrum beta-lactamase-producing *E. coli*. In 78% of UTI episodes colony counts were ≥ 10^5 CFU/mL. ASB was caused by *E. coli* in 14 cases (78%), other pathogens included *Klebsiella* spp., *Enterococcus faecalis* and streptococci.

Median urine leukocyte values

Median urine leukocyte values in UTI patients and controls are displayed in **Table 2**. UTI patients had higher median leukocyte levels compared to control patients with both quantification methods (automated microscopy: 900 versus 26 leukocytes/µl ($p < 0.001$), and urine flowcytometry 1575 versus 23 leukocytes/µl ($p < 0.001$)). Moreover, median leukocyte values were higher for UTI patients than for ASB patients (automated microscopy: 900 versus 296 leukocytes/µl ($p = 0.002$), urine flowcytometry 1575 versus 197 leukocytes/µl ($p = 0.004$)), although interquartile ranges of these groups overlap.

Table 2: Median urine leukocyte values of UTI patients and controls (with subgroups), measured by automated microscopy and urine flowcytometry.

	UTI group	**Control group**		
	UTI (n = 56)	**ASB** (n = 18)	**Neg. culture** (n = 24)	**Mixed flora** (n = 57)
Automated microscopy in cells/µl, median (IQR)	900 (430 – 900)	296 (49 – 773)	4 (1 – 30)	18 (5 – 57)
	UTI (n = 35)	**ASB** (n = 17)	**Neg. culture** (n = 24)	**Mixed flora** (n = 58)
Urine flowcytometry in cells/µl, median (IQR)	1575 (581 – 4673)	197 (43 – 1368)	6 (1 – 35)	20 (4 – 88)

All values are expressed as median (IQR) as leukocyte values did not follow a normal distribution. The UTI column contains both lower and upper UTI patients. Urine flowcytometry data was missing for 28 UTI patients as they were included in regional hospitals in which urine flowcytometry was not available. For automated microscopy values, 900 cells/µl was the upper limit of detection. Abbreviations: UTI = urinary tract infection, ASB = asymptomatic bacteriuria

Diagnostic accuracy

ROC curves for automated microscopy and urine flowcytometry are displayed in **Figure 2A** and **Figure 2B** and contingency tables for sensitivity and specificity calculations are shown in **Supplementary Tables 1A and 1B**. AUC was 0.93 for both diagnostic methods. At a threshold of 264 leukocytes/µl, sensitivity of automated microscopy was 88% (95% CI 77% - 94%) and specificity was 88% (95% CI 80% - 93%), corresponding with a positive likelihood ratio (LR) of 7.2 and a negative LR of 0.1. For urine flowcytometry, sensitivity was 91% (95% CI 79% - 98%) and specificity was 86% (95% CI 78% - 92%) at a cut-off value of 231 leukocytes/µl, with a positive LR of 6.5 and a negative LR of 0.1. Diagnostic accuracy parameters for several theoretical pyuria thresholds are shown in **Table 3**. Applying the currently used cut-off of 10 leukocytes/µl resulted in a sensitivity of 100% (95% CI 94% - 100%) and specificity of 36% (95% CI 28% - 48%). Diagnostic accuracy remained adequate in the sensitivity analysis (**Supplement 2**). The secondary analysis showed that symptomatic patients with mixed flora or ≥ 2 uropathogens all had urine leukocyte counts above our 'optimal' pyuria threshold (264 leukocytes/µl), and all but two patients with negative cultures had counts below this threshold (**Supplement 2**).

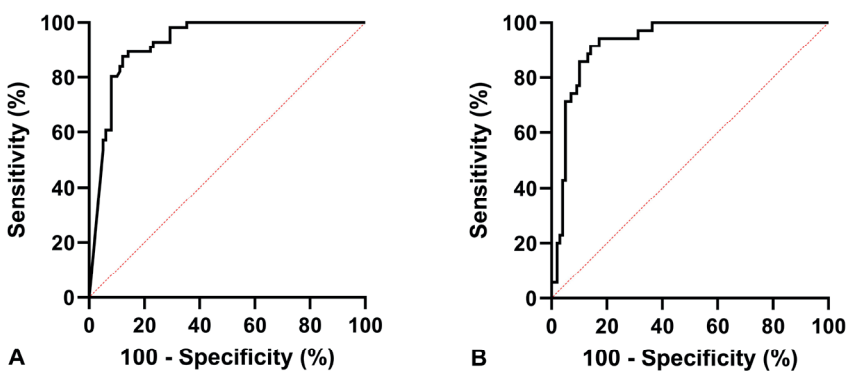


Figure 2: Receiver operating characteristic curves for automated microscopy (A) and urine flowcytometry (B). For both diagnostic methods, the number of leukocytes (per µl) was used as the test variable, and our stringent UTI definition was used for determining disease status. The true positive rate (sensitivity) was plotted against the false positive rate (1 – specificity) for different pyuria cut-offs. The area under the curve was 0.93 for both methods. The reference line is represented by the dotted line.

Table 3: Sensitivity, specificity, and positive and negative likelihood ratios of theoretical pyuria thresholds for diagnosing UTI in older women.

	10 cells/µl	50 cells/µl	100 cells/µl	200 cells/µl	300 cells/µl	400 cells/µl
Sensitivity % (95% CI)	100 (94 – 100)	98 (92 – 100)	93 (84 – 98)	89 (80 – 96)	84 (73 – 92)	77 (65 – 87)
Specificity % (95% CI)	36 (28 – 48)	66 (56 – 75)	71 (61 – 79)	86 (78 – 92)	88 (81 – 93)	92 (86 – 96)
LR_{pos} (95% CI)	1.6 (1.4 – 1.9)	2.9 (2.2 – 3.8)	3.2 (2.3 – 4.3)	6.3 (3.9 – 10.3)	6.9 (4.0 – 11.9)	9.5 (4.8 – 18.7)
LR_{neg} (95% CI)	0.0 (0.0 – 0.1)	0.03 (0.004 – 0.2)	0.1 (0.04 – 0.3)	0.1 (0.06 – 0.3)	0.2 (0.1 – 0.3)	0.3 (0.2 – 0.4)

Diagnostic accuracy parameters are based on automated microscopy results. The currently used cut-off value for pyuria is 10 leukocytes/µl. Abbreviations: LR_{pos} = positive likelihood ratio, LR_{neg} = negative likelihood ratio, CI = confidence interval

Discussion

This explorative study has two important findings. Firstly, we show that the degree of pyuria – quantified by automated microscopy or urine flowcytometry – can help to distinguish UTI in older women from asymptomatic controls, including ASB. Secondly, we demonstrate that the currently used cut-off for pyuria (10 leukocytes/µl) has a very low specificity for UTI in older women, and therefore should not be applied to this population.

Leukocyte counts in UTI

Thus far, the degree of pyuria in UTI and ASB has not been assessed specifically in women aged 65 and over, while a discriminative biomarker is arguably most needed in this population, due to the high prevalence of ASB. In our study, older women with symptomatic UTI had high median urine leukocyte counts (900 and 1575 leukocytes/µl with automated microscopy and urine flowcytometry, respectively). Both Pieretti et al. [18] and Kim et al. [19] quantified pyuria with urine flowcytometry in men and women of all ages, although no separate leukocyte values were given for older patients. Among patients with positive urine cultures, they found median urine leukocyte values of 117 leukocytes/µl and 189 leukocytes/µl, respectively. However, neither of these studies collected clinical data, so misclassification is likely. The discrepancy between urine leukocyte values between these studies and our cohort is likely explained by the fact that we only included cases that met our strict UTI criteria.

Leukocyte counts in ASB

In our study, women with ASB had median counts of 296 leukocytes/µl. Cai et al. [20] included premenopausal women with ASB and a history of recurrent UTI and quantified pyuria with direct microscopy. At baseline, these patients had median urine leukocyte values of 19 per hpf, which corresponds to approximately 100 leukocytes/µl. [21, 22]. This study suggests that higher degrees of pyuria, well above 10 leukocytes/µl, do not necessarily mean that a patient has a UTI, even in premenopausal women. Moreover, urine leukocyte values increased to 54 per hpf (approximately 250 leukocytes/µl) if women developed LUTS during the study and had a positive urine culture. This is in line with our findings that the degree of pyuria is higher in symptomatic patients with positive urine cultures.

Diagnostic accuracy of microscopy and flowcytometry

The majority of UTI studies investigating the discriminative ability of automated microscopy and urine flowcytometry are limited by the absence of a reference standard for UTI. As a consequence, these studies choose a positive urine culture as the reference test, while this does not discriminate between UTI and ASB. Instead, Foudraine et al. [23] defined UTI with an expert panel, taking symptoms and urine culture results into account. They found that automated microscopy had a sensitivity of 86% and a specificity of 82% at a cut-off value of 74 leukocytes/µl. As their study population was younger and antibiotic pretreatment was common, possibly explaining lower pyuria levels, results may not be directly comparable to our study. Diagnostic accuracy parameters are influenced by the studied population, more specifically, how cases and controls are defined. Our control group did not only consist of asymptomatic women with negative urine cultures but rather represents the distribution of urine culture results in asymptomatic older women. For example, the prevalence of ASB in our control group (18%) is very similar to the prevalence of ASB in community-dwelling older women. [4]

Leukocyte counts in symptomatic patients with mixed flora

Our case group only consisted of clear-cut UTI patients fulfilling our stringent criteria. However, urine leukocyte levels were also determined in the ‘suspected UTI’ patients that had new-onset LUTS, but were excluded from the primary analysis because they did not meet our culture criteria. Intriguingly, all excluded patients with either mixed flora or two uropathogens had leukocyte levels above our ‘optimal’ pyuria threshold and all but two patients with negative urine

cultures had levels below that threshold. The finding that all symptomatic patients with mixed flora had high degrees of pyuria, suggests that these patients might have had a true UTI. This is supported by a study showing that over 90% of symptomatic women with *E. coli* as part of mixed flora in their midstream urine cultures actually had *E. coli* bladder bacteriuria as demonstrated by single catheterisation. [9]

Clinical implications

In asymptomatic controls, median urine leukocyte values were higher than the most commonly used cut-off value of 10 leukocytes/µl. Therefore, applying the current pyuria threshold to older women leads to misclassification of many of these women, both with and without ASB. This has several consequences. Firstly, the true cause of the symptoms (e.g. vaginal atrophy, Candida vulvovaginitis, and overactive bladder) remains unidentified and thus untreated if symptoms are wrongfully attributed to UTI. Secondly, it leads to overprescription of antimicrobials, contributing to gut dysbiosis, side effects and selection of resistant pathogens. Gupta et al. [24] show that 25% of asymptomatic patients with pyuria on routine preoperative urinalysis (without urine cultures) were treated with antimicrobials, and that the degree of pyuria predicted prescribing of antimicrobials. These findings, combined with our own data, imply that separate, higher reference values are needed for older women with regards to pyuria. For instance, a threshold of 300 leukocytes/µl would be a considerable improvement, increasing specificity to avoid overtreatment, while still maintaining a fair sensitivity. As in any diagnostic test, pyuria levels should be interpreted within the clinical context of individual patients and should not be the only deciding factor when diagnosing UTI. Since both older women with UTI and asymptomatic older women have a high pretest probability of pyuria, and leukocyte esterase activity is a very rough estimate of the absolute number of leukocytes in the urine [21], the role of urine dipsticks in older patients should, at best, be limited to ruling out UTI. Besides clinical implications, there are also implications for research, as misclassification influences the validity of UTI studies.

Strengths and limitations

Strengths of our study include the use of a stringent UTI definition instead of urine culture as a reference standard, the consistency of results across two quantification

methods (identical AUCs), inclusion of participations from multiple settings, and the rapid analysis of urine samples, increasing reliability of results. Our study has several limitations. Results may not be generalisable to institutionalised older people with high frailty and/or advanced dementia. However, our population was chosen to prove a concept for which a clear definition and reliable assessment of UTI and ASB was deemed necessary. Moreover, our control group contained a higher proportion of LTCF residents than our UTI group. Nonetheless, ADL dependency scores were similar between the UTI and control groups, and median leukocyte values within the LTCF subgroup were comparable to the values of the overall group.

Conclusion

In conclusion, the degree of pyuria should be taken into account when evaluating older women for UTI. Current pyuria cut-offs for UTI are too low and promote inappropriate UTI diagnosis in this population, affecting patient care, antimicrobial stewardship efforts and research. The impact of higher cut-off values on prescription behaviour and UTI related outcomes in older women deserves further study.

Funding

This work was supported by the Netherlands Organisation for Health Research and Development (ZonMw) [grant number 10150511910054]. Sysmex Corporation kindly provided reagents for flowcytometry measurements, but was not involved in the study design or any other study-related processes.

Author contributions

Conceptualisation and methodology M.P.B., J.E.S., C.N., M.E.N., W.P.A., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; recruitment M.P.B., M.J.A., M.M.C.; writing – original draft preparation M.P.B.; data interpretation M.P.B., M.M.C.L., L.G.V.; writing – review and editing M.P.B, M.J.A., E.A., J.E.S., C.N., E.M.S., N.M.D., M.S., M.E.N., W.P.A., S.P.M., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; supervision M.M.C.L. and L.G.V. All authors have read and agreed to the final version of the manuscript.

Acknowledgements

The authors would like to thank Brenda Elzer and Lenneke Vonk for their contribution to participant recruitment. Furthermore, we thank the following inclusion sites: Huisartsenpraktijk de Doelder (Barbara de Doelder), Huisartsenpraktijk Meskers (Angelique Meskers-van Geel), Huisartsenpraktijk Hubertusduin (Maaike Lunstroot, Marja Koppejan), Marente van Wijckerslooth (Johan Verloop, Els van Dijk), WZH Sammersbrug (Jens van Leeuwen), WZH Het Anker (Sander van den Haselkamp), Topaz Overrhyn (Fleur van Zuylen-Jongejan), Topaz Revitel (Sylvia van der Drift-Verbree).

Conflicts of interest

None of the authors have an association that might pose a conflict of interest.

References

1. Ahmed H, Farewell D, Jones HM, Francis NA, Paranjothy S, Butler CC. Incidence and antibiotic prescribing for clinically diagnosed urinary tract infection in older adults in UK primary care, 2004-2014. PLoS One 2018; 13(1): e0190521.
2. Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 2006; 50(6): 1306-14; discussion 14-5.
3. Nicolle LE, Gupta K, Bradley SF, et al. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis 2019; 68(10): 1611-5.
4. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis 2008; 40(10): 804-10.
5. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc 1995; 43(7): 772-5.
6. Mitchell SL, Shaffer ML, Loeb MB, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med 2014; 174(10): 1660-7.
7. Rotjanapan P, Dosa D, Thomas KS. Potentially inappropriate treatment of urinary tract infections in two Rhode Island nursing homes. Arch Intern Med 2011; 171(5): 438-43.
8. Boscia JA, Abrutyn E, Levison ME, Pitsakis PG, Kaye D. Pyuria and asymptomatic bacteriuria in elderly ambulatory women. Ann Intern Med 1989; 110(5): 404-5.
9. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 2013; 369(20): 1883-91.
10. Mabeck CE. Studies in urinary tract infections. IV. Urinary leucocyte excretion in bacteriuria. Acta Med Scand 1969; 186(3): 193-8.
11. Stamm WE. Measurement of pyuria and its relation to bacteriuria. Am J Med 1983; 75(1B): 53-8.
12. Oyaert M, Delanghe J. Progress in Automated Urinalysis. Ann Lab Med 2019; 39(1): 15-22.
13. Hooton TM, Roberts PL, Stapleton AE. Asymptomatic Bacteriuria and Pyuria in Premenopausal Women. Clin Infect Dis 2021; 72(8): 1332-8.
14. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20): 2191-4.
15. Bellelli G, Morandi A, Davis DH, et al. Validation of the 4AT, a new instrument for rapid delirium screening: a study in 234 hospitalised older people. Age Ageing 2014; 43(4): 496-502.
16. Katz S. Assessing self-maintenance: activities of daily living, mobility, and instrumental activities of daily living. J Am Geriatr Soc 1983; 31(12): 721-7.
17. Cobbaert CM, Arslan F, Caballe Martin I, et al. Automated urinalysis combining physicochemical analysis, on-board centrifugation, and digital imaging in one system: A multicenter performance evaluation of the cobas 6500 urine work area. Pract Lab Med 2019; 17: e00139.

18. Pieretti B, Brunati P, Pini B, et al. Diagnosis of bacteriuria and leukocyturia by automated flow cytometry compared with urine culture. J Clin Microbiol 2010; 48(11): 3990-6.

19. Kim H, Kim HR, Kim TH, Lee MK. Age-Specific Cutoffs of the Sysmex UF-1000i Automated Urine Analyzer for Rapid Screening of Urinary Tract Infections in Outpatients. Ann Lab Med 2019; 39(3): 322-6.

20. Cai T, Lanzafame P, Caciagli P, et al. Role of increasing leukocyturia for detecting the transition from asymptomatic bacteriuria to symptomatic infection in women with recurrent urinary tract infections: A new tool for improving antibiotic stewardship. Int J Urol 2018; 25(9): 800-6.

21. van den Broek D, Keularts IM, Wielders JP, Kraaijenhagen RJ. Benefits of the iQ200 automated urine microscopy analyser in routine urinalysis. Clin Chem Lab Med 2008; 46(11): 1635-40.

22. Ichiyanagi Y. Field Volume of Urine Sediment Test - Comparison of Theoretical Volume with Practical Volume. Sysmex Journal International 2014; 24(1).

23. Foudraine DE, Bauer MP, Russcher A, et al. Use of Automated Urine Microscopy Analysis in Clinical Diagnosis of Urinary Tract Infection: Defining an Optimal Diagnostic Score in an Academic Medical Center Population. J Clin Microbiol 2018; 56(6).

24. Gupta K, O'Brien W, Gallegos-Salazar J, Strymish J, Branch-Elliman W. How Testing Drives Treatment in Asymptomatic Patients: Level of Pyuria Directly Predicts Probability of Antimicrobial Prescribing. Clin Infect Dis 2020; 71(3): 614-21.

Supplement

Supplementary Table 1A: Cross tabulation of automated microscopy results against reference standard.

	UTI	Control	Total
Positive (≥ 264 leukocytes/µl)	49	12	61
Negative (< 264 leukocytes/µl)	7	87	94
Total	56	99	155

Index test results (automated microscopy) are displayed in the left column. For calculation of sensitivity/specificity and likelihood ratios, all values below 264 leukocytes/µl were considered negative, and all values of 264 leukocytes/µl and higher were considered positive

Supplementary Table 1B: Cross tabulation of urine flowcytometry results against reference standard.

	UTI	Control	Total
Positive (≥ 231 leukocytes/µl)	32	14	46
Negative (< 231 leukocytes/µl)	3	85	88
Total	35	99	134

Index test results (urine flowcytometry) are displayed in the left column. For calculation of sensitivity/specificity and likelihood ratios, all values below 231 leukocytes/µl were considered negative, and all values of 231 leukocytes/µl and higher were considered positive.

Supplement 2

Sensitivity analysis

In the best-case scenario, i.e. missing automated microscopy data being either true positive or true negative, sensitivity and specificity were 89% (95% CI 80% – 95%) and 88% (95% CI 81% - 94%) respectively. In the worst-case scenario, sensitivity and specificity were 78% (95% CI 67% - 87%) and 86% (95% CI 79% - 92%).

Secondary analysis of cases not meeting culture criteria

Of the 27 patients who were not included in the primary analysis, four patients did not have pyuria. All four of these patients had negative urine cultures, strongly suggesting that their symptoms were caused by a condition other than UTI. The remaining 23 patients did have pyuria but had either mixed flora or ≥ 2 uropathogens (n = 9), or negative cultures (no growth or growth of non-pathogenic micro-organisms, n = 14). Urine leukocyte levels were available for 20/23 (87%) patients. The remaining three patients either had only dipstick results available (leukocyte esterase positive) or pyuria could not be reliably quantified due to macroscopic haematuria. Median urine leukocyte values were 900 leukocytes/µl (IQR 745 -900) in patients with mixed flora or ≥ 2 uropathogens, and 89 leukocytes/µl (IQR 42 – 187) in patients with negative cultures. All patients with mixed flora or ≥ 2 uropathogens had leukocyte counts above our 'optimal' pyuria threshold of 264 leukocytes/µl, and all but two patients with negative cultures had leukocyte counts below the optimal pyuria threshold.

Chapter 5

Diagnostic accuracy of urine biomarkers for urinary tract infection in older women: a case-control study

Manu P. Bilsen, Maxim M. Treep, Margaretha J. Aantjes, Esther van Andel, Janneke E. Stalenhoef, Cees van Nieuwkoop, Eliane M.S. Leyten, Nathalie M. Delfos, Janneke I.M. van Uhm, Martijn Sijbom, Abimbola A. Akintola, Mattijs E Numans, Wilco P. Achterberg, Simon P. Mooijaart, Martha T. van der Beek, Christa M. Cobbaert, Simon P. Conroy, Leo G. Visser, Merel M.C. Lambregts

Clin Microbiol Infect. 2023 Oct 5:S1198-743X(23)00488-3

Abstract

Objectives

Urinary tract infection (UTI) is common among older women. However, diagnosis is challenging due to frequent chronic lower urinary tract symptoms (LUTS), cognitive impairment, and a high prevalence of asymptomatic bacteriuria (ASB). Current urine diagnostics lack specificity, leading to unnecessary treatment and antimicrobial resistance. This study aimed to evaluate the diagnostic accuracy of twelve urine biomarkers for diagnosing UTI in older women.

Methods

In this case-control study, cases were women ≥ 65 years with ≥ 2 new-onset LUTS, pyuria and one uropathogen ≥ 10^4 CFU/mL. Controls were asymptomatic and classified as ASB (one uropathogen ≥ 10^5 CFU/mL), negative culture or mixed flora. Urine biomarker concentrations were measured through liquid chromatography-mass spectrometry and ELISA. Diagnostic accuracy parameters of individual biomarkers and a biomarker model were derived from ROC curves.

Results

We included 162 community-dwelling and institutionalised older women. Five urine inflammatory biomarkers demonstrated high discriminative ability (AUC ≥ 0.80): interleukin 6 (IL-6), azurocidin, neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinases 2 (TIMP-2), and C-X-C motif chemokine 9 (CXCL-9). Azurocidin exhibited the highest diagnostic accuracy (sensitivity 86% (95% confidence interval (CI) 75-93%) and specificity 89% (95% CI 82-94%) at 16.7 ng/mmol creatinine). A combined biomarker and pyuria model showed improved diagnostic accuracy in UTI and ASB patients, compared to pyuria alone.

Conclusions

We identified several urine biomarkers that accurately differentiated older women with UTI from asymptomatic women, including ASB. These findings represent a potential advancement towards improved diagnostics for UTI in older women and warrant validation in a diverse population.

Introduction

Urinary tract infection (UTI) is the second most common infection requiring hospitalisation among older adults and the most common infection in long-term care facility (LTCF) residents. [1, 2] In older women particularly, diagnosing UTI is challenging for various reasons. Firstly, symptom assessment is hampered by a higher prevalence of cognitive impairment and indwelling catheters. Secondly, chronic lower urinary tract symptoms (LUTS), e.g. urgency, frequency and urinary incontinence, are common and are difficult to distinguish from non-infectious causes, such as genitourinary syndrome of menopause, and overactive bladder. [3] Furthermore, up to 50% of non-catheterised older women have asymptomatic bacteriuria (ASB), of which 90% have concomitant pyuria. [4-8] Hence, the specificity of the most commonly used diagnostics for UTI (leukocyte esterase or nitrite on dipstick and urine cultures) is low in this population. [9] Especially in patients with non-specific symptoms, clinicians are inclined to test for and treat bacteriuria and pyuria, which are easily misclassified as UTI. [10] This potentially inappropriate treatment can contribute to antimicrobial resistance, unnecessary side effects and drug interactions in a population with already high rates of polypharmacy. Moreover, it may promote gut dysbiosis and *Clostridioides difficile* infections. [10-14]

As highlighted by the Infectious Diseases Society of America (IDSA), antimicrobial stewardship begins with diagnostic stewardship, and novel biomarkers with high specificity for UTI are urgently needed to endorse prudent use of antibiotics for UTI in older women. [4] Beyond improving individual patient management, an accurate urine biomarker or biomarker panel would also have implications for clinical trial design, drug development, infection surveillance and infection control efforts. A number of studies have evaluated the diagnostic accuracy of several urine inflammatory markers in patients with UTI and ASB, as summarised in a recent systematic review. [15] However, the majority of the included studies either involved younger patients or defined UTI based on dipstick or urine culture results, and are likely affected by misclassification bias. The primary aim of this study was to assess the diagnostic accuracy of twelve urine biomarkers associated with inflammation and tissue injury, for diagnosing UTI in older women. The selection of these biomarkers was based on a review of the available literature and their theoretical potential if no prior evidence was available. [15-21]

Methods

Study design

This multicentre, prospective, case-control study was conducted across four primary care offices, five emergency departments (one academic and four regional hospitals), four LTCFs, and 14 independent and assisted living facilities in the Leiden and The Hague area in the Netherlands. Details of the study design have been published previously. [8] The study protocol was approved by the regional medical ethics committee and written informed consent was obtained from all participants. This study was registered at the International Clinical Trials Registry Platform (trial ID: NL9477) and is reported in accordance with STARD guidelines. [22]

Participants

Cases consisted of women ≥ 65 years meeting all of the following criteria: ≥ 2 new-onset LUTS (dysuria, frequency, urgency, or suprapubic pain), and pyuria (either ≥ 10 leukocytes/µl or the presence of leukocyte esterase on dipstick), and a urine culture with growth of one uropathogen ≥ 10^4 colony-forming units per millilitre (CFU/mL). Uropathogens included Enterobacterales, enterococci, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, and streptococci. Cases with growth of two or more pathogens were excluded. If fever was present (temperature ≥ 38.0 °C), cases were categorised as having an upper UTI. Controls were women ≥ 65 years without new-onset LUTS or fever. Based on urine culture results, they were subdivided into an ASB group (two consecutive urine cultures, obtained 2-4 weeks apart, with identical uropathogens ≥ 10^5 CFU/mL [4]), a 'negative culture' group (no growth or growth of non-pathogenic micro-organisms < 10^3 CFU/mL), or a 'mixed flora' group (≥ 2 pathogens ≥ 10^3 CFU/mL). Exclusion criteria for both cases and controls included: inability to express symptoms (e.g. due to advanced cognitive impairment), the presence of an indwelling catheter, immunosuppressive drug use, antimicrobial use within 48 hours prior to inclusion, current urolithiasis, and a UTI in the previous month.

Procedures

The research team was notified by the attending physician upon identifying a prospective participant. Asymptomatic LTCF residents were invited to participate by their attending physician, while flyers were used to recruit community-dwelling controls. Eligible cases were visited by the research team within one

hour of identification. During the baseline assessment, data on age, previous medical history, new-onset symptoms, and fever were collected. All participants underwent delirium screening and activities of daily living (ADL) assessment using 4AT and Katz questionnaires, and measurement of vital signs.

Midstream urine (or urine obtained through single in-out catheterisation) was collected in a sterile urine container and transported to the laboratory of the Leiden University Medical Center. Samples were transported at room temperature and processed within 4 hours of micturition. (Pre)analytical procedures of urinalysis and microbiological assessments are described elsewhere. [8] In preparation of biomarker analysis, urine was transferred into a 15 mL collection tube and centrifuged (3000 *g* for 8 minutes). The supernatant was transferred into another collection tube and vortexed. Finally, the urine was divided into six aliquots (300 µl per aliquot) and stored at -80 °C until in-batch analysis. Samples underwent no more than a single freeze-thaw cycle.

Biomarker measurements

Biomarker measurements were performed by our in-house developed and validated multiplex liquid chromatography mass spectrometry (LC-MS) with modifications [23] and enzyme-linked immunosorbent assay (ELISA). The following biomarkers were measured using LC-MS: neutrophil gelatinase-associated lipocalin (NGAL), insulin-like growth factor-binding protein 7 (IGFBP-7), tissue inhibitor of metalloproteinases 2 (TIMP-2), kidney injury molecule 1 (KIM-1), C-X-C motif chemokine 9 (CXCL-9), nephrin, solute carrier family 22 member 2 (SLC22A2), calbindin, and transforming growth factor beta-1 (TGF-β1). ELISA was used to measure interleukin 6 (IL-6), xanthine oxidase (XO), and azurocidin (also known as heparin-binding protein). Details on the LC-MS and ELISA analyses are described in the **Supplementary Material**.

Sample size calculation

As sensitivity and specificity values of urine biomarkers were either conflicting or unknown for our population, we assumed sensitivity and specificity values for our sample size calculation. To assess specificity, with an α of 0.05, and with maximum marginal error of estimate of 0.10 (δ) for constructing the confidence interval (CI) of the true value of specificity, assuming a value of 80% and using the normal approximation, the control group needed to consist of 62 participants.

Using the same sample size for the case group resulted in a marginal error (δ) of sensitivity, assuming a true value of 70%, of 0.12.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM, Armonk, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A Mann-Whitney U test was performed to compare median biomarker concentrations between cases and controls, and a Bonferroni-corrected significance level (α) of 0.005 was applied. Sensitivity-specificity pairs were computed for all possible thresholds and plotted in a receiver operating characteristic (ROC) curve using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California). To determine the discriminative ability of each urine biomarker, we calculated the area under the curve (AUC) for the individual biomarkers. The continuous variable CXCL9 was dichotomised as it was undetectable in many participants. 'Optimal' cut-offs for each biomarker were based on Youden's J statistic, and two additional cut-offs were calculated for scenarios in which either a sensitivity of 90% or a specificity of 90% was desired. To investigate whether these biomarkers performed better in combination, we fitted a logistic regression model using backward selection which included all (logarithmically transformed) biomarkers, selected on Akaike's Information Criterion. The AUC of this regression model was compared with the AUC of the best performing individual biomarker using DeLong's test.

We recently published data demonstrating that the degree of pyuria can be helpful in distinguishing UTI in older women from asymptomatic controls, including those with ASB. [8] To investigate the additional value of the biomarkers, we conducted a post hoc analysis comparing the discriminative ability of a model containing both urinary leukocytes and the biomarker panel with urinary leukocytes alone, using DeLong's test. Given that controls in the ASB subgroup showed intermediate levels of pyuria in our previous study (interquartile ranges overlapped with UTI cases) [8], the same comparison was made in a subset of patients with either UTI or ASB.

Results

Between June 2021 and July 2022, 162 participants were enrolled (screening process summarised in **Figure 1**). Participant characteristics are outlined in **Table 1**. Cases and controls were similar in age, comorbidities and ADL-dependency (38/162

participants (23%) were dependent for ≥ 2 Katz-items). Controls were recruited more often in a LTCF (43/100, 43%) compared with cases (7/62, 11%). Twenty-one percent (13/62) of cases had an upper UTI and 18% (18/100) of controls had ASB. Causative pathogens are summarised in **Supplementary Table 1**; *E. coli* was the most common pathogen in both cases (50/62, 81%) and controls with ASB (14/18, 78%).

Table 1: Baseline characteristics of cases and controls.

Baseline characteristics	UTI (n = 62)	Controls (n = 100)
Age in years, mean (SD)	77.2 (8.0)	79.0 (8.1)
Setting		
Emergency department	18 (29.0)	0
LTCF	7 (11.3)	43 (43.0)
Primary care office	37 (60.0)	0
At home	0	57 (57.0)
Comorbidity		
Urological comorbidity	8 (12.9)	8 (8.0)
Diabetes mellitus	14 (22.6)	14 (14.0)
History of CKD (self-reported)	12 (19.4)	11 (11.0)
ADL-dependency ≥ 2 Katz-items	14 (22.6)	24 (24.0)
UTI history		
Ever had UTI	56 (90.3)	76 (76.0)
Ever hospitalised for UTI	2 (3.2)	1 (1.0)
No. of UTI in past year, median (IQR)	1 (0 – 2)	0 (0 – 0)
Antibiotics in previous month	16 (25.8)	20 (20.0)
Catheter in week prior to inclusion	2 (3.2)	2 (2.0)
New-onset symptoms	62 (100)	0
Dysuria	48 (77.4)	-
Frequency	56 (90.3)	-
Urgency	52 (83.9)	-
Suprapubic pain	42 (67.7)	-
Fever (≥ 38.0)	13 (21.0)	-
4AT score ≥ 2	4 (6.5)	1 (1.0)

Variables are expressed as n (%) unless otherwise specified. Urological comorbidity included pelvic organ prolapse, previous procedures for urinary incontinence and previous malignancies (n = 1 renal cell carcinoma, n = 1 non-muscle-invasive bladder cancer; no evidence of active malignancy in either patient). All participants with a 4AT score ≥ 2 were able to communicate their symptoms clearly. UTI = urinary tract infection, LTCF = long-term care facility, CKD = chronic kidney disease, ADL = activities of daily living

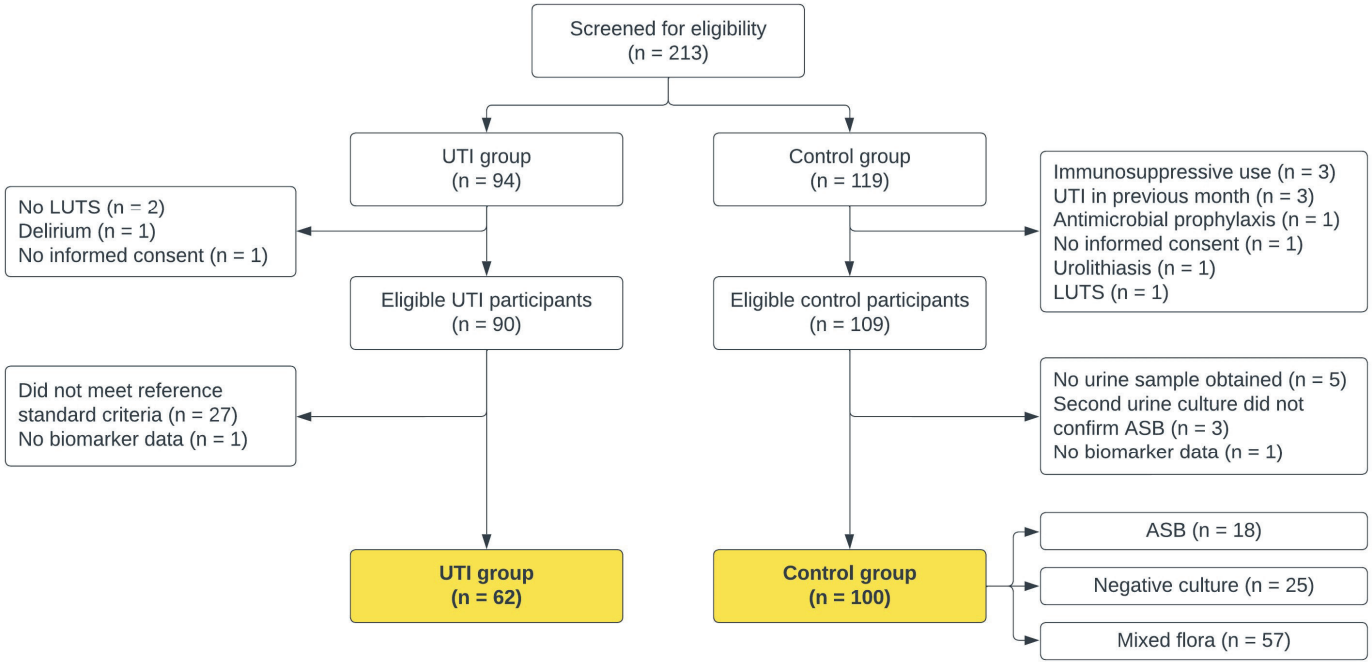


Figure 1: Overview of screening and selection process. The 27 participants that did not meet reference standard criteria were symptomatic patients who did not have pyuria or urine cultures with growth of 1 uropathogen. For 2 participants, biomarker data was missing. UTI = urinary tract infection, LUTS = lower urinary tract symptoms, ASB = asymptomatic bacteriuria

Biomarker concentrations and diagnostic accuracy

Median urine biomarker concentrations for cases and controls are shown in **Figure 2** and **Supplementary Table 2**. LC-MS biomarkers nephrin, SLC22A2, and TGF-β1 were not detected in any participant. Except for uromodulin and calbindin, all biomarkers differed significantly between cases and controls. CXCL-9 was detected in 40/62 (65%) cases and 5/100 (5%) controls (χ^2 67.6, p < 0001).

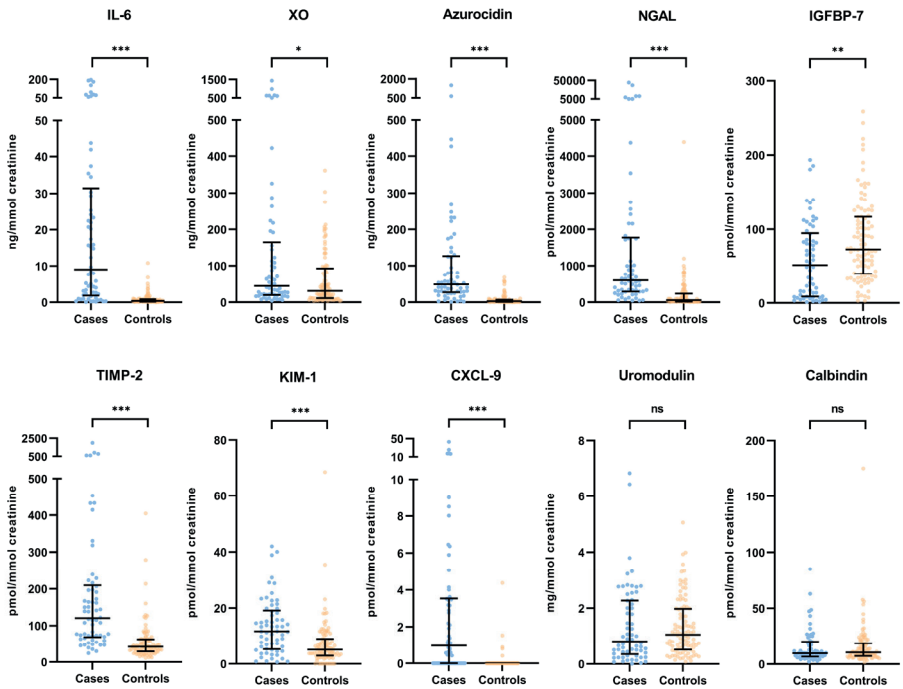


Figure 2: Scatter dot plots of biomarker concentrations for cases and controls. The horizontal line drawn in the middle denotes the median, and the whiskers represent the interquartile range. Significance levels are indicated by: ns = not significant, * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Abbreviations: IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7 (IGFBP-7), TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1 CXCL-9 = C-X-C motif chemokine 9.

ROC curves and corresponding AUCs are displayed in **Figure 3**. IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 all had excellent discriminative ability (AUC ≥ 0.80). Sensitivity, specificity and likelihood ratios for various cut-offs are shown in

Table 2. IL-6 (cut-off 1.88 ng/mmol creatinine) and azurocidin (cut-off 16.7 ng/mmol creatinine) had high specificity (90% (95% CI 83-95%) and 89% (95% CI 82-94%), respectively), while maintaining fair sensitivity (76% (95% CI 64-85%) and 86% (95% CI 75-93%), respectively). After backward selection, our logistic regression model (ROC curve in **Figure 3** and model summary in **Supplementary Table 5**) contained the following biomarkers: IL-6, XO, azurocidin, NGAL, TIMP-2, CXCL-9 and uromodulin. This model had better discriminative ability (AUC 0.95) than the biomarker with the highest AUC in the univariate analysis (azurocidin, AUC 0.92), albeit not statistically significant (p = 0.06).

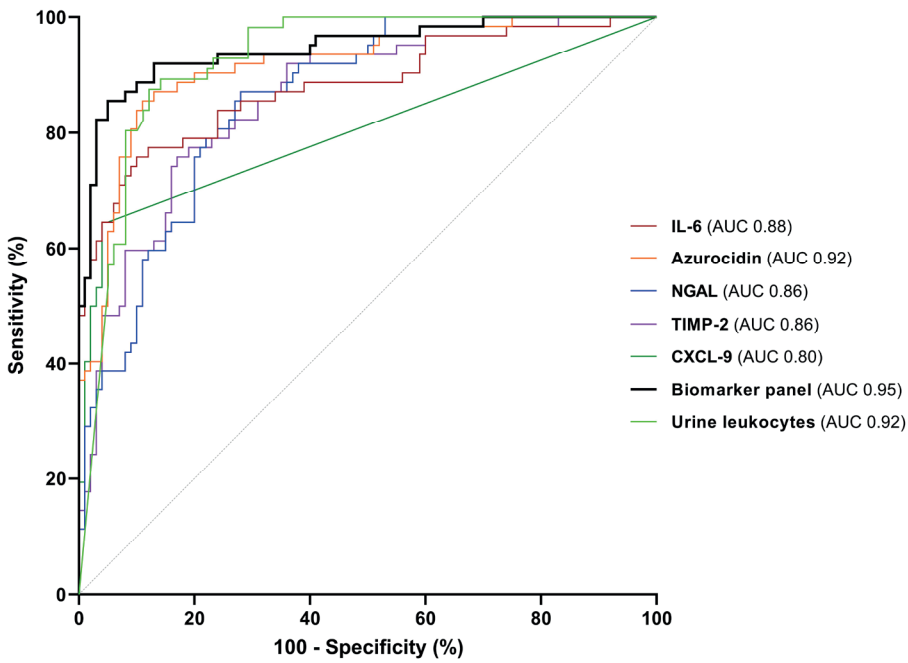


Figure 3: Receiver operating characteristic curves for IL-6, azurocidin, NGAL, TIMP-2, CXCL-9 and a combined biomarker model. Biomarker concentrations were used as test variables, and our UTI definition was used for determining disease status. The true positive rate (sensitivity) was plotted against the false positive rate (1 – specificity) for different biomarker cut-offs. Our combined logistic regression model contained the following logarithmically transformed biomarkers: IL-6, XO, azurocidin, NGAL, TIMP-2, CXCL-9 and uromodulin. Areas under the curve were: IL-6 (0.88), azurocidin (0.92), NGAL (0.86), TIMP-2 (0.86), CXCL-9 (0.80), combined biomarker model (0.95). The ROC curve of CXCL-9 is diagonal due to ties between cases and controls, i.e. CXCL-9 concentration was 0 in some of cases and controls. The reference line is represented by the dotted line. Abbreviations: IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, CXCL-9 = C-X-C motif chemokine 9.

Table 2: Diagnostic accuracy parameters of IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 for various cut-offs.

	Cut-off	Sensitivity % (95%CI)	Specificity % (95%CI)	LR_{pos} (95% CI)	LR_{neg} (95% CI)
IL-6 (ng/mmol creatinine) optimal	1.88	76 (64 – 85)	90 (83 – 95)	7.6 (4.1 – 13.9)	0.3 (0.2 – 0.4)
High sensitivity preferred	0.28	90 (81 – 96)	43 (34 – 53)	1.6 (1.3 – 1.9)	0.2 (0.1 – 0.5)
High specificity preferred	1.88	76 (64 – 85)	90 (83 – 95)	7.6 (4.1 – 13.9)	0.3 (0.2 – 0.4)
Azurocidin (ng/mmol creatinine) optimal	16.7	86 (75 – 93)	89 (82 – 94)	7.8 (4.4 – 13.7)	0.2 (0.09 – 0.3)
High sensitivity preferred	8.7	90 (81 – 96)	80 (72 – 97)	4.5 (3.0 – 6.7)	0.1 (0.05 – 0.3)
High specificity preferred	17.0	84 (73 – 92)	90 (83 – 95)	8.4 (4.6 – 15.3)	0.2 (0.1 – 0.3)
NGAL (pmol/mmol creatinine) optimal	201	87 (77 – 94)	72 (63 – 80)	3.1 (2.2 – 4.3)	0.2 (0.09 – 0.3)
High sensitivity preferred	115	90 (81 – 96)	63 (53 – 72)	2.4 (1.9 – 3.2)	0.2 (0.07 – 0.3)
High specificity preferred	598	50 (38 – 62)	90 (83 – 95)	5.0 (2.6 – 9.5)	0.6 (0.4 – 0.7)
TIMP-2 (pmol/mmol creatinine) optimal	69.7	76 (64 – 85)	83 (75 – 89)	4.4 (2.8 – 7.0)	0.3 (0.2 – 0.5)
High sensitivity preferred	47.1	90 (81 – 96)	64 (54 – 73)	2.5 (1.9 – 3.3)	0.2 (0.07 – 0.3)
High specificity preferred	89.4	60 (47 – 71)	90 (83 – 95)	6.0 (3.2 – 11.1)	0.4 (0.3 – 0.6)
CXCL-9 (pmol/mmol creatinine)	Present or absent	65 (52 – 75)	95 (90 – 98)	12.9 (5.4 – 30.9)	0.4 (0.3 – 0.5)

The optimal cut-off value was based on Youden's J statistic, and two additional cut-offs were calculated for scenarios in which either a sensitivity of 90% or a specificity of 90% was desired. CXCL9 was dichotomised as it was undetectable in a large number of patients. IL-6 = interleukin 6, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, CXCL-9 = C-X-C motif chemokine 9.

Post hoc and subgroup analyses

Overall, the model combining the biomarker panel and urinary leukocytes did not perform significantly better than urinary leukocytes alone; both showed high diagnostic accuracy (AUC 0.95 vs. 0.92). In the subset of patients with either UTI or ASB, the combined biomarker and leukocyte model demonstrated higher diagnostic accuracy (AUC 0.89) compared with urinary leukocytes alone (AUC 0.73), p = 0.01. This effect was also observed for the combination of CXCL9 and leukocytes (AUC 0.86, p = 0.04), but not for other biomarker-leukocyte combinations. Median urine biomarker concentrations for case and control subgroups are detailed in **Supplementary Tables 3 and 4**.

Discussion

In this study, we identified five urine biomarkers with high diagnostic accuracy for UTI in older women. Urinary IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 accurately differentiated older women with UTI from asymptomatic women, including those with ASB. These findings advance the development of better diagnostics for UTI in older women.

Comparison with previous studies

Most urine biomarker research has been performed in children. [16, 24] A few studies have investigated the diagnostic performance of IL-6, azurocidin and NGAL in (older) adults. IL-6 is secreted by urothelial cells following pathogen exposure, and induces an acute phase response. [25] Azurocidin and NGAL are neutrophil-granule derived proteins that exhibit their antibacterial effect through monocyte chemotaxis and sequestration of siderophore-bound iron, respectively. [26, 27] Our findings regarding IL-6 and azurocidin are consistent with findings from previous studies. Kjölvmark et al. [18] observed significantly higher levels of IL-6 and azurocidin in community-dwelling and institutionalised patients with UTI compared with LTCF residents with ASB. Median urinary IL-6 and azurocidin concentrations were similar to concentrations found in our study, although IL-6 concentrations were even higher in their UTI group, possibly due to a higher proportion of upper UTI patients. Rodhe et al. [19] also found significantly higher urinary IL-6 levels in older patients with UTI compared to those with ASB. Both studies only compared UTI and ASB. We deliberately compared patients with UTI to asymptomatic controls (including ASB), as this is the primary distinction to be made in clinical practice, given that urine culture results are not available

at the time of presentation. The diagnostic accuracy of NGAL was previously demonstrated by Price et al. [20], who reported an even higher AUC, likely due to their control group being younger and lacking patients with ASB. CXCL-9, a chemokine that differentiates pyelonephritis from cystitis in children [21], was detected in the majority of UTI patients but only in 5% of controls. Notably, CXCL-9 was undetectable in all 1443 middle-aged participants in a prior LC-MS reference value study [23], supporting the biomarker's high specificity. We did not find any study evaluating the diagnostic accuracy of TIMP-2 for UTI.

Biomarker panel

In clinical practice, pyuria is often assessed when diagnosing UTI. Our recent study showcased that the degree of pyuria can aid in differentiating UTI from asymptomatic controls. [8] The biomarkers evaluated in our current study displayed comparably high diagnostic accuracy. An additional value of the biomarker panel lies in the distinction between UTI and ASB, as urinary leukocyte counts showed some overlap in our previous study. [8] Our post hoc analysis showed that a combination of urine biomarkers and leukocytes had a significantly higher diagnostic accuracy in this subgroup than urine leukocytes alone. Particularly in cases with intermediate degrees of pyuria, this panel could assist the clinician in deciding whether to initiate empirical treatment or not.

Strengths and limitations

The strengths of this study include the implementation of robust and standardised (pre)analytical procedures, ensuring reliable biomarker results. Additionally, we employed strict criteria to define UTI, included three control subgroups, and recruited older women from diverse healthcare settings. However, there are certain limitations to acknowledge. Firstly, the study primarily involved a relatively healthy older population, which may restrict the generalisability of our findings to a more frail population. However, given the absence of an agreed-upon reference standard for UTI, the selection of distinct cases and controls was necessary to identify promising biomarkers warranting further validation. Secondly, we did not measure serum creatinine levels, which prevented us from exploring this potential relationship in our study. [17] As with any case-control study, there is a possibility of overestimated diagnostic accuracy parameters and unmeasured confounding. Lastly, we acknowledge minor differences between cases and controls regarding baseline characteristics. However, additional regression analysis (not shown) did not

demonstrate an effect of age, diabetes mellitus or ADL-dependency on biomarker concentrations.

Conclusions

In conclusion, we have identified five urine biomarkers that exhibit high diagnostic accuracy for UTI in older women: IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9. Moreover, a biomarker panel showed additional value, on top of pyuria, for discriminating UTI from ASB. The performance of these biomarkers needs to be prospectively validated in a broader population with various clinical presentations (including non-specific symptoms), comorbidities and levels of frailty. Future research should then focus on whether the implementation of this diagnostic tool, for instance as a point-of-care test, improves individual patient management, infection surveillance and control efforts, combats antimicrobial resistance, and reduces misclassification bias in UTI studies.

Funding

This work was supported by the Netherlands Organisation for Health Research and Development (ZonMw) [grant number 10150511910054].

Author contributions

Conceptualisation and methodology M.P.B., J.E.S., C.N., J.I.M.U., A.A.A., M.E.N., W.P.A., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; recruitment M.P.B., M.J.A., J.I.M.U., M.M.C.L.; laboratory analysis M.M.T, E.A., C.M.C; writing – original draft preparation M.P.B.; data interpretation M.P.B., M.M.C.L., L.G.V.; writing – review and editing M.P.B, M.M.T., M.M.J.A., E.A., J.E.S., C.N., E.M.S., N.M.D., J.I.M.U, M.S., A.A.A., M.E.N., W.P.A., S.P.M., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; supervision M.M.C.L. and L.G.V. All authors have read and agreed to the final version of the manuscript.

Acknowledgements

The authors would like to acknowledge Renee Ruhaak and Fred Romijn for coordinating and carrying out the laboratory analysis in the Leiden University Medical Center. Moreover we thank Hanneke Engel (Alrijne ziekenhuis), Piet Sturm (Haaglanden Medisch Centrum), John Hagenaar (Haga ziekenhuis), Margret de Koning (Onze Lieve Vrouwe Gasthuis) for coordinating the laboratory analysis in the peripheral hospitals. Furthermore, the authors thank Rolf Groenwold and Jelle Goeman for their contribution to the statistical analysis. Finally, we would like to thank the following collaborators for their contribution to participant recruitment: Brenda Elzer (Leiden University Medical Center), Lenneke Vonk (Haaglanden Medisch Centrum), Barbara de Doelder (Huisartsenpraktijk Hoekstra en de Doelder), Angelique Meskers-van Geel (Huisarstenpraktijk Meskers), Maaike Lunstroot and Marja Koppejan (Huisartsenpraktijk Hubertusduin), Johan Verloop and Els van Dijk (Marente van Wijckerslooth) Jens van Leeuwen (WZH Sammersbrug), Sander van den Haselkamp (WZH Het Anker), Fleur van Zuylen-Jongejan (Topaz Overrhyn), Sylvia van der Drif-Verbree (Topaz Revitel).

Potential conflicts of interest (also mentioned in ICMJE forms)

M.M.C.L. reports grants or contracts as the principal investigator on the Embrace Study. L.G.V. reports grants or contracts as the co-investigator on the Embrace

Study. J.E.S. reports consulting fees from Viiv Expert Board HIV, unrelated to this manuscript; payment or honoraria from Nederlandse Internisten Vereniging: Centraal Onderwijs Interne Geneeskunde Infection and Immunity (a course for internists in training); and participation as the Chair of Dutch Infection Prevention Guideline Committee "Urinary Catheterisation." M.E.N. reports payment for expert testimony for the development of guidelines for primary and secondary care, focusing on GERD and Dyspepsia, Ondansetron, and Non-Alcoholic Fatty Liver Disease, and is a committee member for these guidelines (paid to author); unpaid membership to Network Academic Primary Care, the Netherlands; the Nederlands Huisartsen Genootschap Primary Care Practice Accreditation Board; and the Advisory Board of SIR Institute for Pharmacy Practice and Policy. S.P.C. reports royalties for textbook editing on geriatric emergency medicine, including urinary tract infections; consulting fees as clinical lead of the UK Frailty Improvement Network; and travel support for teaching or speaking on geriatric care across Europe. C M.C. serves as Chair of the International Federation of Clinical Chemistry and Laboratory Medicine Scientific Division (independent).

References

1. Tsan L, Langberg R, Davis C, et al. Nursing home-associated infections in Department of Veterans Affairs community living centers. Am J Infect Control **2010**; 38(6): 461-6.
2. Curns AT, Holman RC, Sejvar JJ, Owings MF, Schonberger LB. Infectious disease hospitalizations among older adults in the United States from 1990 through 2002. Arch Intern Med **2005**; 165(21): 2514-20.
3. Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol **2006**; 50(6): 1306-14; discussion 14-5.
4. Nicolle LE, Gupta K, Bradley SF, et al. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis **2019**; 68(10): 1611-5.
5. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis **2008**; 40(10): 804-10.
6. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc **1995**; 43(7): 772-5.
7. Boscia JA, Abrutyn E, Levison ME, Pitsakis PG, Kaye D. Pyuria and asymptomatic bacteriuria in elderly ambulatory women. Ann Intern Med **1989**; 110(5): 404-5.
8. Bilsen MP, Aantjes MJ, van Andel E, et al. Current Pyuria Cutoffs Promote Inappropriate Urinary Tract Infection Diagnosis in Older Women. Clin Infect Dis **2023**; 76(12): 2070-6.
9. Joseph A. The Diagnosis and Management of UTI in >65s: To Dipstick or Not? The Argument Against Dipsticks. Infect Prev Pract **2020**; 2(3): 100063.
10. Mitchell SL, Shaffer ML, Loeb MB, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med **2014**; 174(10): 1660-7.
11. Rotjanapan P, Dosa D, Thomas KS. Potentially inappropriate treatment of urinary tract infections in two Rhode Island nursing homes. Arch Intern Med **2011**; 171(5): 438-43.
12. Worby CJ, Schreiber HLt, Straub TJ, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol **2022**; 7(5): 630-9.
13. Langford BJ, Brown KA, Diong C, et al. The Benefits and Harms of Antibiotic Prophylaxis for Urinary Tract Infection in Older Adults. Clin Infect Dis **2021**; 73(3): e782-e91.
14. Das R, Towle V, Van Ness PH, Juthani-Mehta M. Adverse outcomes in nursing home residents with increased episodes of observed bacteriuria. Infect Control Hosp Epidemiol **2011**; 32(1): 84-6.
15. Edwards G, Seeley A, Carter A, et al. What is the Diagnostic Accuracy of Novel Urine Biomarkers for Urinary Tract Infection? Biomark Insights **2023**; 18: 117727192211144459.
16. Horvath J, Wullt B, Naber KG, Koves B. Biomarkers in urinary tract infections - which ones are suitable for diagnostics and follow-up? GMS Infect Dis **2020**; 8: Doc24.

17. van Duijl TT, Soonawala D, de Fijter JW, Ruhaak LR, Cobbaert CM. Rational selection of a biomarker panel targeting unmet clinical needs in kidney injury. Clin Proteomics **2021**; 18(1): 10.

18. Kjolvmark C, Tschernij E, Oberg J, Pahlman LI, Linder A, Akesson P. Distinguishing asymptomatic bacteriuria from urinary tract infection in the elderly - the use of urine levels of heparin-binding protein and interleukin-6. Diagn Microbiol Infect Dis **2016**; 85(2): 243-8.

19. Rodhe N, Lofgren S, Strindhall J, Matussek A, Molstad S. Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria. Scand J Prim Health Care **2009**; 27(2): 74-9.

20. Price JR, Guran L, Lim JY, et al. Neutrophil Gelatinase-Associated Lipocalin Biomarker and Urinary Tract Infections: A Diagnostic Case-Control Study (NUTI Study). Female Pelvic Med Reconstr Surg **2017**; 23(2): 101-7.

21. Shaikh N, Martin JM, Hoberman A, et al. Host and Bacterial Markers that Differ in Children with Cystitis and Pyelonephritis. J Pediatr **2019**; 209: 146-53 e1.

22. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ **2015**; 351: h5527.

23. van Duijl TT, Ruhaak LR, Hoogeveen EK, et al. Reference intervals of urinary kidney injury biomarkers for middle-aged men and women determined by quantitative protein mass spectrometry. Ann Clin Biochem **2022**; 59(6): 420-32.

24. Boon HA, Struyf T, Bullens D, Van den Bruel A, Verbakel JY. Diagnostic value of biomarkers for paediatric urinary tract infections in primary care: systematic review and meta-analysis. BMC Fam Pract **2021**; 22(1): 193.

25. Ching CB, Gupta S, Li B, et al. Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. Kidney Int **2018**; 93(6): 1320-9.

26. Linder A, Soehnlein O, Akesson P. Roles of heparin-binding protein in bacterial infections. J Innate Immun **2010**; 2(5): 431-8.

27. Shields-Cutler RR, Crowley JR, Miller CD, Stapleton AE, Cui W, Henderson JP. Human Metabolome-derived Cofactors Are Required for the Antibacterial Activity of Siderocalin in Urine. J Biol Chem **2016**; 291(50): 25901-10.

Supplement

Supplementary methods: biomarker analysis and quality control

ELISA

ELISA analyses were carried out using the Quantikine™ human IL-6 kit (R&D Systems, Minneapolis, MN, Art. No. HS600C), the XO kit (Cusabio, Houston, TX, Art. No. CSB-E13124h), and the human azurocidin kit (Cusabio, Houston, TX, Art. No. CSB-E09698h). All analyses were executed in accordance with the provided manuals and quality controls were performed for each kit. The IL-6 ELISA kit performance was tested with low, medium and high concentration quantitative controls purchased from R&D systems (Quantikine™, Immunoassay Control Group 246, Cat. No. QC246). All quantitative controls passed the predefined criteria provided by the manufacturer. In addition, an in-house prepared urine pool of healthy individuals was used as an internal quality control. Finally, two in-house prepared single-donor (kidney transplantation patient) samples were used for quality assurance, as no quality controls were provided by the manufacturer. Average coefficients of variation (CV) were 6.3% (IL-6), 20.1% (XO), and 13.9% (AZU). Lower limits of detection were 0.03 pg/mL (IL-6), 0.04 ng/mL (XO) and 2.0 pg/mL (azurocidin), respectively. If the upper limit of detection was reached, samples were diluted as prescribed by the manual. Final biomarker concentrations were normalised for urinary dilution using creatinine (mmol/L) and reported in ng/mmol creatinine. Creatinine concentrations were determined for each sample with an enzymatic assay using a Cobas C502 (Roche Diagnostics, Rotkreuz, Switzerland).

LC-MS

The other nine biomarkers were analysed with our in-house developed and validated multiplex LC-MS test with modifications. The use of alternative antibodies for calbindin (R&D systems AF3320; Polyclonal Goat IgG) and TGF-β1 (R&D systems BAF240; Polyclonal Chicken IgY) improved the measuring range and increased sensitivity by 10-fold. For KIM-1, TIMP-2, CXCL-9 and TGF-β1 optimised LC-MS settings were used. The optimised method was employed to measure the samples in a total of three batches in 96-well format including five urine-based calibrators and two urine-based internal quality controls in duplicate per batch, for the purpose of quantification and quality assurance, respectively. The performance of the LC-MS instrument passed the criteria of the system suitability test that was run prior to and after each sample batch. Internal quality controls for all three

batches passed the predefined criteria. Specifically, the average CVs for QC1 and QC2 were 13.7% and 15.4%, respectively. LC–MS biomarker concentrations (pmol/L) were normalised for urinary dilution and reported in pmol/mmol creatinine.

Supplementary Table 1: List of causative pathogens in cases and controls with asymptomatic bacteriuria (ASB).

	Cases (n = 62)	**Controls with ASB (n = 18)**
Escherichia coli n (%)	50 (81)	14 (79)
Klebsiella spp. n (%)	3 (5)	2 (11)
Proteus mirabilis n (%)	3 (5)	0
Citrobacter (non) koseri n (%)	2 (3)	0
Enterococcus faecalis/faecium n (%)	2 (3)	1 (6)
Pseudomonas aeruginosa n (%)	2 (3)	0
Group C *Streptococcus* n (%)	0	1 (6)

In two cases, *Escherichia coli* isolates produced extended-spectrum beta-lactamase. In all controls with ASB, we required growth of identical pathogens in two consecutive urine cultures, obtained two to four weeks apart, with at least 10^5 colony-forming units per millilitre. *Klebsiella* spp. included *Klebsiella pneumoniae* (n = 4) and *Klebsiella oxytoca* (n = 1).

Supplementary Table 2: Median urine biomarker concentrations for cases and controls.

	Cases (n = 62)	Controls (n = 100)	Unadjusted P-value	AUC (95%CI)
IL-6 ng/mmol creatinine, median (IQR)	9.0 (1.9 – 31.4)	0.34 (0.16 – 0.83)	< 0.001	0.88 (0.82 – 0.94)
XO ng/mmol creatinine, median (IQR)	44.3 (19.6 – 164.6)	30.8 (11.2 – 92.7)	0.04	0.60 (0.51 – 0.69)
Azurocidin ng/mmol creatinine, median (IQR)	48.4 (27.1 – 126.5)	2.6 (0.90 – 6.9)	< 0.001	0.92 (0.87 – 0.96)
NGAL pmol/mmol creatinine, median (IQR)	594 (289 – 1772)	59 (20 – 234)	< 0.001	0.86 (0.80 – 0.91)
IGFBP-7 pmol/mmol creatinine, median (IQR)	51.3 (8.7 – 94.6)	72.4 (39.2 – 117.0)	0.002	0.65 (0.56 – 0.74)
TIMP-2 pmol/mmol creatinine, median (IQR)	120 (69 – 209)	42 (29 – 63)	< 0.001	0.86 (0.80 – 0.92)
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (5.3 – 19.1)	5.2 (3.0 – 9.0)	< 0.001	0.72 (0.64 – 0.80)
CXCL-9 pmol/mmol creatinine, median (IQR)	0.98 (0 – 3.5)	0 (0 – 0)	< 0.001	0.80 (0.72 – 0.88)
Uromodulin mg/mmol creatinine, median (IQR)	0.82 (0.35 – 2.27)	1.06 (0.52 – 1.98)	0.38	0.54 (0.45 – 0.64)
Calbindin pmol/mmol creatinine, median (IQR)	9.8 (6.7 – 20.3)	10.5 (7.3 – 18.9)	0.65	0.52 (0.43 – 0.62)

All values are normalised for urinary dilution. A Mann-Whitney U test was performed to compare median biomarker concentrations between cases and controls. Uncorrected p-values are shown, we applied a Bonferroni-corrected significance level (α) of 0.005. The area under the curve (AUC) of each individual biomarker was derived from the receiver operating characteristic curve of each biomarker. Abbreviations: IQR = interquartile range, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 3: Summary of the logistic regression model with a combination of biomarkers obtained through backward selection.

	Beta	Odds ratio (95%CI)	P-value
XO	-1.11	0.33 (0.09 – 1.13)	0.09
Azurocidin	1.38	3.96 (1.22 – 15.23)	0.03
NGAL	1.16	3.19 (0.75 – 16.07)	0.13
TIMP-2	-2.38	0.09 (0.004 – 1.88)	0.13
IL-6	1.97	7.19 (1.78 – 35.14)	0.009
CXCL-9	1.66	5.27 (1.61 – 20.53)	0.01
Uromodulin	-1.30	0.27 (0.05 – 1.45)	0.14

The R package MASS was used for backwards variable selection. All variables in this model were logarithmically transformed, due to the large variance observed in some of these biomarkers. Abbreviations: XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, IL-6 = interleukin 6, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 4: Median urine biomarker concentrations for lower versus upper UTI.

	Lower UTI (n = 49)	**Upper UTI (n = 13)**	**Unadjusted P-value**
IL-6 ng/mmol creatinine, median (IQR)	5.2 (1.1 – 27.2)	23.3 (13.6 – 50.1)	0.046
XO ng/mmol creatinine, median (IQR)	32.6 (17.7 – 98.0)	192.0 (35.6 – 560.8)	0.02
Azurocidin ng/mmol creatinine, median (IQR)	47.5 (28.3 – 127.9)	49.3 (16.0 – 163.4)	0.72
NGAL pmol/mmol creatinine, median (IQR)	576 (287 – 1790)	610 (265 – 2990)	0.94
IGFBP-7 pmol/mmol creatinine, median (IQR)	34.3 (8.1 – 84.8)	82.0 (37.8 – 146.6)	0.03
TIMP-2 pmol/mmol creatinine, median (IQR)	115 (62 – 202)	151 (73 – 271)	0.30
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (4.8 – 19.8)	13.4 (6.8 – 16.2)	0.97
CXCL-9 pmol/mmol creatinine, median (IQR)	1.08 (0 – 3.56)	0.82 (0.21 – 4.97)	0.79
Uromodulin mg/mmol creatinine, median (IQR)	0.75 (0.39 – 2.08)	0.91 (0.19 – 2.67)	0.95
Calbindin pmol/mmol creatinine, median (IQR)	8.8 (6.0 – 12.3)	26.0 (9.9 – 45.4)	0.001

All values are normalised for urinary dilution. A Mann-Whitney U test was performed to compare median biomarker concentrations between lower and upper UTI patients. P-values not corrected for multiple testing are shown. Abbreviations: UTI = urinary tract infection, IQR = interquartile range, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 5: Median biomarker concentrations for control subgroups.

	UTI group	Control group				
	UTI (n = 62)	**ASB (n = 18)**	**Neg. culture (n = 25)**	**Mixed flora (n = 57)**	**P-value***	**AUC****
IL-6 ng/mmol creatinine, median (IQR)	9.0 (1.9 – 31.4)	0.65 (0.18 – 2.19)	0.20 (0.15 – 0.49)	0.39 (0.15 – 0.82)	< 0.001	0.82
XO ng/mmol creatinine, median (IQR)	44.3 (19.6 – 164.6)	27.5 (9.2 – 63.4)	39.1 (15.6 – 82.5)	34.9 (11.8 – 99.0)	0.054	0.65
Azurocidin ng/mmol creatinine, median (IQR)	48.4 (27.1 – 126.5)	6.4 (2.3 – 20.2)	1.3 (0.8 – 3.4)	2.8 (0.8 – 6.4)	< 0.001	0.82
NGAL pmol/mmol creatinine, median (IQR)	594 (289 – 1772)	320 (129 – 699)	23 (11 – 73)	55 (21 – 219)	0.03	0.67
IGFBP-7 pmol/mmol creatinine, median (IQR)	51.3 (8.7 – 94.6)	54.1 (28.6 – 72.8)	95.8 (50.2 – 152.4)	79.6 (39.8 – 115.8)	0.92	0.51
TIMP-2 pmol/mmol creatinine, median (IQR)	120 (69 – 209)	44 (35 – 131)	42 (28 – 58)	41 (27 – 59)	0.002	0.74
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (5.3 – 19.1)	7.0 (3.7 – 12.5)	3.5 (1.6 – 6.7)	5.3 (3.0 – 8.7)	0.13	0.62
CXCL-9 pmol/mmol creatinine, median (IQR)	0.98 (0 – 3.5)	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	< 0.001	0.80
Uromodulin mg/mmol creatinine, median (IQR)	0.82 (0.35 – 2.27)	1.05 (0.32 – 1.39)	0.84 (0.38 – 2.72)	1.13 (0.65 – 2.00)	0.85	0.51
Calbindin pmol/mmol creatinine, median (IQR)	9.8 (6.7 – 20.3)	13.7 (7.0 – 24.6)	9.4 (7.2 – 17.1)	10.8 (7.2 – 19.1)	0.43	0.56

*P-value is shown for comparison UTI versus ASB, using a Mann-Whitney U test. **Area under the receiver operating characteristic curve is shown for discriminating UTI from ASB. All values are normalised for urinary dilution. Abbreviations: UTI = urinary tract infection, ASB = asymptomatic bacteriuria, AUC = area under the curve, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.

Part III

Alternative prophylactic and treatment strategie

Chapter 6

Intravesical aminoglycoside instillations as prophylaxis for recurrent urinary tract infection: patient satisfaction, long-term safety and efficacy

Manu P. Bilsen, Janneke I.M. van Uhm, Janneke E. Stalenhoef, Cees van Nieuwkoop, Rolf H.H. Groenwold, Leo G. Visser, Merel M.C. Lambregts

JAC Antimicrob Resist. 2023 Apr 6;5(2):dlad040

Abstract

Background

Recurrent urinary tract infections (UTI) are common, especially in women. When oral antimicrobial prophylaxis is ineffective or not possible due to allergies or antimicrobial resistance, intravesical aminoglycoside instillations (IAI) are a non-systemic alternative.

Objectives

To assess treatment satisfaction, long-term safety and efficacy of IAI for recurrent UTI.

Methods

We conducted a cohort study using data collected between January 2013 and June 2022 at the Leiden University Medical Center. Adult patients with recurrent UTI who received prophylactic IAI were eligible for inclusion. Treatment satisfaction was assessed through a survey. Data on serum aminoglycoside concentrations, cystoscopy results, and number of recurrences were obtained through chart review. Number of recurrences and UTI characteristics were compared between patients on and off IAI using Poisson and logistic mixed effects models.

Results

Forty-four patients were included (median follow-up time 976 days) and 323 UTIs occurred during follow-up. Overall treatment satisfaction was high (median 79.2/100). All but one patient had undetectable serum aminoglycoside levels and no malignancies were found on follow-up cystoscopy. IAI increased the time to first recurrence (102 days versus 36 days, p = 0.02), reduced the number of recurrences (RR 0.75, 95%CI 0.56 – 0.99, p = 0.04), and the necessity for systemic antibiotics (OR 0.33, 95%CI 0.13 – 0.86, p = 0.02).

Conclusions

In patients with recurrent UTI, IAI was associated with high treatment satisfaction, and was found to be a safe and effective alternative to oral antimicrobial prophylaxis.

Introduction

Recurrent urinary tract infection (UTI) refers to at least three episodes per year or two episodes per 6 months. [1] While morbidity of a single UTI is low, the high incidence and recurrence risk lead to considerable healthcare costs and a reduced quality of life. [2, 3] In patients with high recurrence rates despite behavioural modifications and non-antimicrobial prophylaxis, oral antimicrobial prophylaxis is often initiated. Continuous antimicrobial prophylaxis reduces recurrence risk, including in patients who perform clean intermittent catheterisation (CIC). [4, 5] However, an important disadvantage of continuous oral antimicrobial prophylaxis is the emergence of resistant pathogens, limiting treatment options. [5, 6] This is especially relevant for patients with an increased risk of infections with multidrug resistant organisms (MDRO), e.g. patients with neurogenic bladder and kidney transplant recipients. [7, 8] In addition to antimicrobial resistance (AMR), allergies and side effects may preclude oral antimicrobial prophylaxis as a viable treatment strategy for recurrent UTI. [9]

In an era where AMR is a rising threat to global health, direct instillation of antibiotics in the bladder may be an appealing alternative to systemic antimicrobial prophylaxis. [10] With intravesical aminoglycoside instillations (IAI), high concentrations of aminoglycosides – which exhibit concentration-dependent killing – are achieved in the bladder. Consequently, uropathogens without high-level aminoglycoside resistance can still be treated with IAI as concentrations in the bladder exceed MIC breakpoints. [11] Systemic uptake of aminoglycosides is rare, diminishing the concern for nephrotoxicity and ototoxicity. [11] As aminoglycosides stay in the bladder, it is hypothesised that the commensal flora of the gut, perineum and vagina may remain unaffected. In fact, Stalenhoef et al. [11] showed a reduction in MDRO UTIs, possibly also explained by a decrease in overall systemic antibiotic use. [12] Treatment satisfaction has not yet been assessed with validated tools. Evaluating treatment satisfaction is particularly relevant for patients receiving IAI, as it is more invasive than other prophylactic alternatives, and treatment satisfaction influences treatment-related behaviour (adherence and persistence), ultimately affecting treatment success. [13] Since the study by Stalenhoef et al. [11], the Leiden University Medical Center (LUMC) has implemented IAI in an increasing number of outpatients with recurrent UTI, most of them continuing IAI after 6 months. As a consequence, more long-term data have become available. The aim of this study is to assess treatment satisfaction, long-term safety and efficacy of IAI in patients with recurrent UTI.

Methods

We conducted a cohort study using data collected between January 2013 and June 2022 in our tertiary care hospital for assessment of long-term safety and efficacy. Treatment satisfaction was assessed through a cross-sectional survey (May 2022). This study was approved by the regional ethics committee (METC-LDD) and all patients provided written informed consent for the use of their data and survey participation. This study was registered at clinicaltrials.gov (NCT05376670).

Study population

Adult recurrent UTI patients who were on continuous or postcoital IAI were eligible for inclusion. Patients exclusively using IAI for on-demand treatment of recurrences (no prophylaxis) were excluded. Moreover, we excluded patients receiving IAI for chronic prostatitis and patients with an indwelling catheter. Patients with multiple treatment cycles (on and off IAI) acted as their own controls.

IAI treatment protocol

Patients received training for CIC and the preparation of the solution by a specialised nurse. They were instructed to mix 80 mg of gentamicin with 20 mL of 0.9% sodium chloride (tobramycin 80 mg or amikacin 250 mg were chosen in case of infections with a gentamicin-resistant pathogen within the preceding 6 months). To increase bladder time, patients were advised to administer the solution before bedtime. The standard treatment regimen consisted of daily instillations for 2 weeks, every other day for 10 weeks, and twice weekly for 12 weeks. In case of new-onset lower urinary tract symptoms (LUTS), daily instillations were reinitiated for 5-7 days if signs of systemic infection were absent. If LUTS persisted or systemic signs were present, oral or intravenous antibiotics were started. Patients were instructed to directly contact the outpatient clinic instead of their general practitioner for all new-onset symptoms, regardless of whether they were on IAI at that time. After 6 months of IAI, discontinuation of treatment was discussed with all patients. If treatment was continued, IAI frequency was individualised and based on recurrence rate. Serum aminoglycoside levels were measured in the first month, after an overnight instillation. Cystoscopy was performed every two years.

Data collection

Clinical data were collected from electronic records and included baseline demographics, comorbidities, other prophylactic measures, and previous MDRO UTIs. For safety endpoints we collected cystoscopy and serum aminoglycoside data. To establish efficacy, we recorded the number of recurrences during follow-up. For each UTI, additional information was collected on LUTS, fever (temperature ≥ 38.0 ºC), microbiological results, hospital admission and treatment.

We defined UTI as an episode with new-onset symptoms that was diagnosed as a UTI by a physician and was treated with an antimicrobial agent. Dysuria, frequency, urgency and suprapubic pain were classified as LUTS, other non-genitourinary symptoms were classified as 'non-specific symptoms'. Both conversion to daily IAI and oral/intravenous antibiotics were considered treatment. We considered ESBL and carbapenemase-producing Enterobacterales, Enterobacterales with combined fluoroquinolone and aminoglycoside resistance, and vancomycin-resistant enterococci as MDRO. MIC-breakpoints for resistance and intermediate sensitivity were based on EUCAST-criteria. [14]

Treatment satisfaction

Treatment satisfaction was only assessed in patients who were on IAI at the time of data collection or had been using IAI no longer than one year before the start of data collection. Treatment satisfaction was assessed through a linguistically-validated Dutch version of the Treatment Satisfaction Questionnaire for Medication-version II (TSQM-II) in a paper format. [15] Permission was obtained from IQVIA Inc. (One IMS Drive, Plymouth Meeting, PA-19462). The TSQM-II consists of 11 questions, divided into four domains: effectiveness, side effects, convenience, and global satisfaction. Scores are calculated by adding items in each domain and transforming the composite score into a value ranging from 0 to 100, where a score of 100 corresponds with the highest degree of satisfaction. For the side effects domain, a score of 100 indicates an absence of side effects.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM, Armonk, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as percentages, means with standard deviations, or medians with IQR based on the type and distribution of the data. To compare UTI characteristics

between patients on and off IAI, a logistic mixed effects model with a varying intercept per patient was used, to take dependencies between observations (recurrences) per patient into account. The Kaplan-Meier method was used to estimate time to first UTI recurrence; results were graphically displayed and compared between patients on IAI and after cessation of IAI using a log-rank test. In case of multiple IAI cycles, only the first IAI cycle was included in the Kaplan-Meier analysis. To compare the incidence of UTI episodes between patients on and off IAI, a Poisson mixed effects model was used (with random intercept per patient). As the duration of treatment cycles markedly varied, 'duration' was log-transformed and included as an offset in the model. For the Poisson model, we assumed that risk of recurrence was constant over time. Since this assumption may not hold true, we performed a sensitivity analysis in which these data were analysed using a Cox frailty model. Prior to data analysis, sample size was calculated for treatment satisfaction. To estimate the mean overall score on the TSQM-II questionnaire with a margin of error indicated by a 95% CI not wider than 20, a sample size of 25 patients was required, given the expected population standard deviation of 25.4. [5] Subgroup analyses were performed based on gender, menopausal status, history of kidney transplantation, and history of CIC prior to IAI. To determine whether effects of IAI treatment differed between subgroups, Poisson mixed effects models with interaction terms were applied.

Results

Patient characteristics

In total, 44 patients were included (inclusion flowchart in **Supplementary Figure 1**). Patient characteristics are outlined in **Table 1**. Most patients in our cohort were postmenopausal women receiving IAI due to failure of oral antimicrobial prophylaxis (57%) or the lack of oral options due to AMR (36%). Twenty-eight patients (68%) were already performing CIC prior to the initiation of IAI and 11 patients (25%) had a history of kidney transplantation. Median follow-up duration was 976 days (IQR 468 – 1637) and median number of IAI days was 602 (IQR 402 – 1212).

Treatment satisfaction and (dis)continuation

At 6 months, 80% of patients wished to continue IAI, because of fewer recurrences and an increased quality of life (self-reported). Two patients discontinued after 6-months due to insufficient efficacy, and one patient was switched to oral

Table 1: Baseline characteristics of patients with recurrent UTI starting IAI.

Baseline characteristics	**n = 44**
Age in years	61.9 (14)
Female	31 (71)
Postmenopausal	25/31 (81)
Sexually active	13/21 (62)
Comorbidity	
Previous CIC	28 (68)
Underactive/neurogenic bladder (including spina bifida)	27 (61)
Kidney transplantation	11 (25)
Urethral dilation/meatal dilation/urethrotomy	10 (23)
Diabetes mellitus	8 (18)
Cystocele/rectocele	7 (16)
Nephrectomy	5 (11)
TURP	5 (11)
ADPKD	3 (7)
Urolithiasis	3 (7)
Urological malignancy	0
eGFR mL/min/1.73 m2 prior to start of IAI	
≥ 90	12 (27)
60 – 89	21 (48)
45 – 59	4 (9)
30 – 44	3 (7)
15 – 29	4 (9)
Non-antimicrobial prophylaxis	
Vaginal oestrogen	22/31 (71)
D-mannose	13 (30)
Non-antibiotic irrigations	11 (25)
UTI caused by MDRO in 6 months before IAI	17 (39)
Indication for IAI	
Oral prophylaxis not efficacious	25 (57)
No oral options due to resistance	16 (36)
No oral options due to intolerance	15 (34)
No oral options due to allergy	4 (9)
Other reason	6 (14)
Frequency of IAI at last follow-up	
Daily	7 (16)
Every other day	10 (23)
Twice weekly	13 (30)
No IAI at last follow-up	14 (32)

Age is expressed as mean (SD); all other variables are expressed as n (%). Sexual activity was not reported for 10 women. Other reasons for initiation of IAI: patient preferred IAI over oral prophylaxis, patient already did CIC and had recurrent urinary tract infections. Abbreviations: IAI = intravesical aminoglycoside instillations, CIC = clean intermittent catheterisation, TURP = transurethral resection of the prostate, ADPKD = autosomal dominant polycystic kidney disease, eGFR = estimated glomerular filtration rate, MDRO = multidrug resistant organism.

prophylaxis because resistance to oral antimicrobial therapy was lost. Of the 26 patients that discontinued IAI at some point during follow-up, 18 (69%) restarted IAI. The TSQM-II was filled out by 32 patients (73%), and results are summarised in **Figure 1**. Median scores of the four domains were: global satisfaction 79.2 (IQR 66.7 – 100.0), effectiveness 83.3 (IQR 66.7 – 97.9), side effects 100.0 (IQR 100.0 – 100.0), and convenience 69.4 (IQR 61.1 – 83.3). Two patients completing the questionnaire reported side effects, being painful CIC. Global satisfaction was higher for patients who were already performing CIC before initiation of IAI compared to patients who did not have prior experience with CIC (median score 83.3 versus 58.3, p = 0.03). Discontinuation rates and TSQM-scores did not differ for the specified subgroups (data not shown).

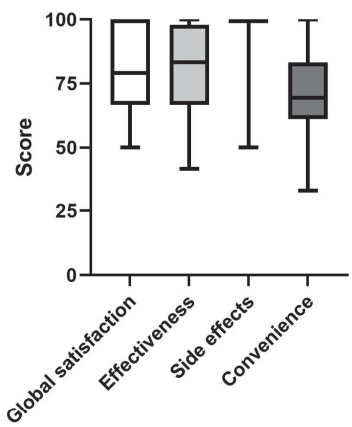


Figure 1: Box and whiskers plot of Treatment Satisfaction Questionnaire for Medication version II (TSQM-II) scores in patients with current or recent IAI treatment (n = 32). Median values are represented by the black line within the boxes; the median value of the side effects domain was 100.

Safety

Cystoscopy was performed in 29 patients (66%) after a median of 768 days (IQR 363 – 1327) since the start of IAI. No malignancies were found. Other cystoscopy findings included bladder trabeculation (n = 6), diverticula (n = 3) and cystitis cystica/glandularis (n = 2). Serum aminoglycoside levels were available for 40 patients (91%). All but one patient had undetectable serum aminoglycoside levels. The patient with a detectable aminoglycoside level (serum tobramycin 0.5 mg/L) had macroscopic haematuria (due to a recent bladder biopsy for a suspected fungal cystitis) at the time of measurement.

Efficacy

Recurrences and antimicrobial consumption

In total, 323 UTIs (207 during IAI prophylaxis, 116 after IAI prophylaxis) were reported in 44 patients. UTI characteristics are outlined in **Table 2**. LUTS were present in 209/268 (78.0%) episodes and fever in 44/323 (13.6%) episodes. Median time to first recurrence was longer for patients on IAI compared to after cessation of IAI (102 days versus 36 days, p = 0.02), as summarised in **Figure 2**. Moreover, IAI significantly decreased the number of recurrences (rate ratio 0.75, 95%CI 0.56 – 0.99, p = 0.04). A positive effect of IAI was also consistently seen in various Cox frailty models (**Supplementary Table 1**). In patients on IAI, 75.2% of recurrences were treated with systemic (oral or intravenous) antibiotics, compared to 92.2% of recurrences after cessation of IAI (OR 0.33, 95%CI 0.13 – 0.86, p = 0.02).

Table 2: Characteristics and treatment of UTIs in patients with IAI and after cessation of IAI.

	IAI n (%)	No IAI n (%)	OR (95%CI)	p-value
New-onset LUTS	122/169 (72.2)	87/99 (87.9)	0.43 (0.16 – 1.18)	0.10
Fever	30/207 (14.5)	14/116 (12.1)	1.23 (0.45 – 3.34)	0.68
UTI caused by classic GNR	101/164 (61.6)	75/102 (73.5)	0.66 (0.31 – 1.43)	0.29
UTI caused by enterococci	26/164 (15.9)	5/102 (4.9)	4.45 (1.40 – 12.88)	0.01
MDRO (including ESBL)	22/155 (14.2)	18/99 (18.2)	0.78 (0.28 – 2.19)	0.64
Hospital admission	30/206 (14.6)	10/116 (8.6)	1.09 (0.34 – 3.56)	0.88
Necessity for systemic (oral/ intravenous) antibiotics	155/206 (75.2)	107/116 (92.2)	0.33 (0.13 – 0.86)	0.02

E. coli, *Proteus mirabilis* and *Klebsiella pneumoniae* were defined as classic Gram-negative rods. Missing data: new-onset LUTS (n = 55), hospital admission (n = 1), necessity for systemic antibiotics (n = 1). In 54 UTI episodes, no urine culture was performed. Odds ratios were calculated using a logistic mixed effects model with a varying intercept per patient. Abbreviations: OR = odds ratio, 95%CI = 95% confidence interval, UTI = urinary tract infection, LUTS = lower urinary tract symptoms, GNR = Gram-negative rods, MDRO = multi-drug resistant organism, ESBL = extended spectrum beta-lactamase.

The results of the subgroup analyses are provided in **Supplementary Table 2**. In the subgroup of women, the time to first recurrence was 98 versus 23 days, p = 0.02 and the rate ratio of recurrences was 0.59 (95%CI 0.43 – 0.81, p = 0.001).

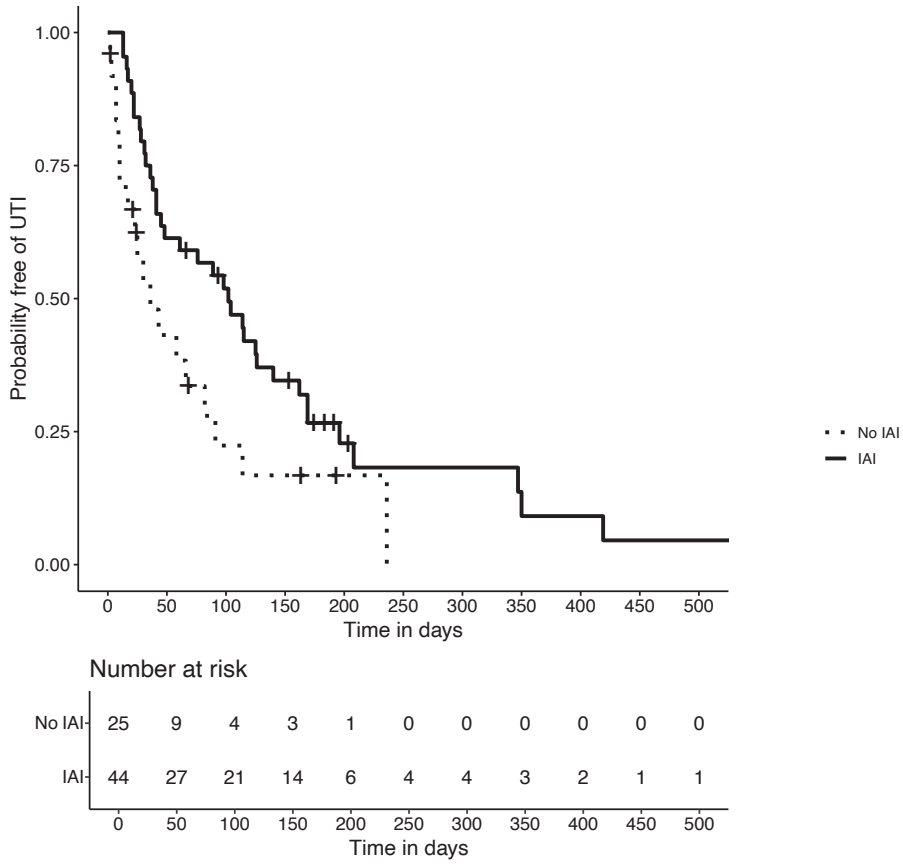


Figure 2: Kaplan-Meier curve of time to first recurrence (UTI) in patients with IAI and after cessation of IAI. Patients on IAI treatment are indicated by the solid line, and patients that have stopped IAI by the dotted line. Abbreviations: IAI = intravesical aminoglycoside instillations.

Microbiological characteristics

A urine culture was performed in 267 episodes (82.7%). In 216 cases (80.9%) a single uropathogen was found, while in 20 cases (7.5%) two uropathogens, in 21 cases (7.9%) mixed flora, and in 10 cases (3.7%) no uropathogens were found. Recurrences that occurred during IAI were more often caused by enterococci than recurrences that occurred after cessation of IAI (OR 4.45, 95%CI 1.40 – 12.88, p = 0.01). No differences were found in the same comparison for classic Gram-negative rods (*E. coli, Klebsiella pneumoniae,* and *Proteus mirabilis*). In the 6 months before initiation of IAI, 17 patients had a UTI caused by an MDRO (5 were aminoglycoside

resistant). Three of these 17 patients experienced a recurrence with the same MDRO in the 6 months after initiation of IAI.

Sensitivity analysis

Eight patients (18%) in our study had also participated in the study by Stalenhoef et al.[11] Including only the remaining 36 patients (82%) in our Poisson model produced a rate ratio of 0.75 (95%CI 0.53 – 1.05). Furthermore, results of our logistic mixed effects model were not affected by missing clinical and microbiological data (**Supplementary Table 3**).

Discussion

In patients with recurrent UTI, IAI is associated with high treatment satisfaction and continuation rates, and it appears to be a safe and effective alternative to oral antimicrobial prophylaxis.

Treatment satisfaction

Thus far, treatment satisfaction for IAI has not been assessed with a validated questionnaire. Stalenhoef et al. [11] requested patients to grade their satisfaction by providing a score between 0 and 10 and found a mean score of 8 (SD 1.2) after 24 weeks of IAI. This score is similar to the overall satisfaction score that was found in our study (median 79.2 out of 100). However, an overall score does not give insight into the different domains of treatment satisfaction. The highest satisfaction scores were observed in the 'effectiveness' and 'side-effects' domains. In fact, only two patients reported any side effects (painful catheterisation). Contrary to previous studies, no gastro-intestinal complaints or vaginal infections were reported. [11, 12] The validated questionnaire that we used in our study was also used in a randomised trial evaluating oral antimicrobial prophylaxis in patients with recurrent UTI and CIC use. [5] Scores for effectiveness were comparable to our IAI cohort. However, convenience scores were lower in our patients with IAI (mean 71.2, SD 16.1) compared to patients in the oral prophylaxis study (mean 88.9, SD 13.9). Lower convenience scores for IAI are unsurprising as CIC is necessary for administration of the drug. In the oral prophylaxis study, all patients were already performing CIC and questions focused on convenience of oral therapy alone.

Safety

Serum aminoglycoside levels were undetectable in all but one patient, confirming results of previous studies that systemic uptake is very rare. [11, 16-18] In treatment of non-muscle-invasive bladder cancer, systemic uptake of intravesical agents occurs more frequently in case of mucosal damage, due to recent transurethral resection, traumatic catheterisation or an active UTI. [19] In an infected rat bladder model, systemic aminoglycoside absorption was observed in 3/7 rats, but serum aminoglycoside levels were all in the non-toxic range. [20] The serum concentration that was found in one patient (0.5 mg/L) was likely related to disruption of the epithelial barrier due to recent bladder biopsies. This concentration is considered non-toxic as it falls below the usual trough levels for systemic aminoglycoside treatment. [21] We propose that routine measurement of serum aminoglycoside concentration should no longer be performed in patients using IAI, except in patients with macroscopic haematuria.

Neither in our study, nor in the study by Stalenhoef et al. [11] were malignancies found on follow-up cystoscopy. Our study had markedly longer follow-up times, with a quarter of patients having a follow-up cystoscopy more than 3.5 years after initiation of IAI. However, caution is warranted when interpreting these findings, as our sample size was relatively small, bladder cancer incidence is generally low, and the median age of our cohort lies below the median age at bladder cancer diagnosis.

Efficacy

In our study, IAI significantly reduced the number of recurrences and necessity for systemic (oral/intravenous) antibiotics. These findings are consistent with previous studies, most of them including patients with neurogenic bladder. [11, 12, 17, 22, 23] In subgroup analyses the effect of IAI seemed to be most pronounced in women, which is in contrast with the results of two previous studies that also investigated the effect of gender. [11, 22] However, caution should be applied when interpreting results of subgroup analyses, as the subgroups were small and other determinants had a skewed distribution. For instance, 54% of men were kidney transplant recipients, compared to 13% of women.

The majority of studies compared the number of recurrences in the 6 months prior to IAI to the number of recurrences in the 6 months after initiation of IAI. However, Stalenhoef et al. [11] showed that recurrence rates in the 6 months after

cessation of IAI remained low. In this study, follow-up started at the initiation of IAI and recurrence rates were compared between on and off IAI cycles, meaning that patients off IAI had already used IAI in the past. It is possible that the reduction in recurrence rate would have been even more pronounced had we compared recurrence rates between patients on IAI and prior to initiation of IAI. A comparison between self-reported recurrence rate (before IAI) to physician-reported recurrence rate was not deemed ideal. In patients receiving IAI, we observed that fewer recurrences had to be treated with systemic antibiotics. This observation underestimates the reduction of the total antibiotic burden, as recurrence rates are also lower in patients with IAI use.

It is incompletely understood which mechanisms contribute to the efficacy of IAI. Worby et al. [24] have shown that gut microbial richness is significantly lower in women with recurrent UTI. In this study, 1 in 4 recurrences were treated with daily IAI only. We hypothesise that a reduction in systemic antibiotic use (due to a decrease in recurrence rate as well as treating recurrences with IAI only) may promote a recovery of a dysbiotic gut microbiome, thereby potentially reducing recurrence risk. Another hypothesis is that IAI may eradicate intracellular bacterial reservoirs that can seed recurrent infection. [25]

Implications for clinical practice

Despite a lower recurrence rate on IAI, breakthrough infections do occur. If signs of systemic infection are absent, primary management with daily IAI is preferable, to avoid the drawbacks of systemic antimicrobials. If symptoms persist despite daily IAI, and systemic antimicrobial therapy is necessary, the different pathogen distribution among IAI-users is relevant for empirical therapy. We observed that most patients who had had a UTI caused by an MDRO in the 6 months prior to IAI did not have a recurrence with that same pathogen. Moreover, recurrences that developed during IAI prophylaxis were more frequently caused by enterococci, which is likely explained by the fact that enterococci are frequently intrinsically resistant to high levels of aminoglycosides.

Strengths and limitations

Strengths of our study include the long follow-up time, the use of a validated questionnaire to assess treatment satisfaction, and the inclusion of subgroup analyses. Furthermore, the results regarding efficacy were consistent across

different statistical approaches. Our study has several limitations. Firstly, the TSQM-II questionnaire was administered at the same time for all patients, which led to a variable timing of the questionnaire in relation to treatment duration. Most respondents were on IAI at the time of the survey, which might have led to an overestimation of treatment satisfaction. Secondly, due to the observational nature of this study we did not use an existing reference standard for UTI, which might have contributed to misclassification of UTI. However, this effect will have occurred in both 'groups' (on and off IAI) and biased results are therefore unlikely. Another limitation is the unblinded nature of this study. Finally, a limitation that is inherent to observational studies is unmeasured confounding.

Conclusion

In conclusion, IAI is a safe and effective non-systemic alternative for UTI prophylaxis with a high degree of treatment satisfaction. It should be considered in patients who fail oral antimicrobial prophylaxis or have allergies and resistance patterns that preclude oral prophylaxis as a viable strategy. Future studies should focus on elucidating the best regimen in terms of dosage and frequency.

Acknowledgements

The authors would like to thank P.H. Verbeek-Menken and I.G.A. Nellen for instructing patients on IAI use. Moreover, we thank B.E.L. Vrijsen for his valuable contribution to the statistical analysis of the data.

Funding

This study was carried out as part of routine work.

Transparency declaration

None of the authors have an association that might pose a conflict of interest.

Author contributions

Conceptualisation and methodology M.P.B., M.M.C.L., L.G.V., and R.H.H.G.; writing – original draft preparation M.P.B.; data interpretation M.P.B., M.M.C.L., L.G.V., and R.H.H.G.; writing – review and editing M.P.B, M.M.C.L., J.I.M.U., J.E.S., C.N., L.G.V.; supervision M.M.C.L. and L.G.V. All authors have read and agreed to the final version of the manuscript.

References

1. Bonkat G, Bartoletti F, Bruyere F, et al. EAU Guidelines on Urological Infections. Available at: https://uroweb.org/guidelines/urological-infections. Accessed 25th of February 2023.
2. Ennis SS, Guo H, Raman L, Tambyah PA, Chen SL, Tiong HY. Premenopausal women with recurrent urinary tract infections have lower quality of life. Int J Urol 2018; 25(7): 684-9.
3. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol 2010; 7(12): 653-60.
4. Beerepoot MA, ter Riet G, Nys S, et al. Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. Arch Intern Med 2011; 171(14): 1270-8.
5. Fisher H, Oluboyede Y, Chadwick T, et al. Continuous low-dose antibiotic prophylaxis for adults with repeated urinary tract infections (AnTIC): a randomised, open-label trial. Lancet Infect Dis 2018; 18(9): 957-68.
6. Beerepoot MA, ter Riet G, Nys S, et al. Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women. Arch Intern Med 2012; 172(9): 704-12.
7. McKibben MJ, Seed P, Ross SS, Borawski KM. Urinary Tract Infection and Neurogenic Bladder. Urol Clin North Am 2015; 42(4): 527-36.
8. Korth J, Kukalla J, Rath PM, et al. Increased resistance of gram-negative urinary pathogens after kidney transplantation. BMC Nephrol 2017; 18(1): 164.
9. Malik RD, Wu YR, Christie AL, Alhalabi F, Zimmern PE. Impact of Allergy and Resistance on Antibiotic Selection for Recurrent Urinary Tract Infections in Older Women. Urology 2018; 113: 26-33.
10. Global antimicrobial resistance surveillance system (GLASS) report: early implementation. Available at: https://apps.who.int/iris/handle/10665/332081.
11. Stalenhoef JE, van Nieuwkoop C, Menken PH, Bernards ST, Elzevier HW, van Dissel JT. Intravesical Gentamicin Treatment for Recurrent Urinary Tract Infections Caused by Multidrug Resistant Bacteria. J Urol 2019; 201(3): 549-55.
12. Cox L, He C, Bevins J, Clemens JQ, Stoffel JT, Cameron AP. Gentamicin bladder instillations decrease symptomatic urinary tract infections in neurogenic bladder patients on intermittent catheterization. Can Urol Assoc J 2017; 11(9): E350-E4.
13. Jacobs JM, Pensak NA, Sporn NJ, et al. Treatment Satisfaction and Adherence to Oral Chemotherapy in Patients With Cancer. J Oncol Pract 2017; 13(5): e474-e85.
14. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2020; Version 10.0.
15. Atkinson MJ, Kumar R, Cappelleri JC, Hass SL. Hierarchical construct validity of the treatment satisfaction questionnaire for medication (TSQM version II) among outpatient pharmacy consumers. Value Health 2005; 8 Suppl 1: S9-S24.
16. Marei MM, Jackson R, Keene DJB. Intravesical gentamicin instillation for the treatment and prevention of urinary tract infections in complex paediatric urology patients: evidence for safety and efficacy. J Pediatr Urol 2021; 17(1): 65 e1- e11.

17. Abrams P, Hashim H, Tomson C, Macgowan A, Skews R, Warren K. The use of intravesical gentamicin to treat recurrent urinary tract infections in lower urinary tract dysfunction. Neurourol Urodyn 2017; 36(8): 2109-16.

18. Defoor W, Ferguson D, Mashni S, et al. Safety of gentamicin bladder irrigations in complex urological cases. J Urol 2006; 175(5): 1861-4.

19. Koya MP, Simon MA, Soloway MS. Complications of intravesical therapy for urothelial cancer of the bladder. J Urol 2006; 175(6): 2004-10.

20. Wan J, Kozminski M, Wang SC, et al. Intravesical instillation of gentamicin sulfate: in vitro, rat, canine, and human studies. Urology 1994; 43(4): 531-6.

21. Bertino JS, Jr., Booker LA, Franck PA, Jenkins PL, Franck KR, Nafziger AN. Incidence of and significant risk factors for aminoglycoside-associated nephrotoxicity in patients dosed by using individualized pharmacokinetic monitoring. J Infect Dis 1993; 167(1): 173-9.

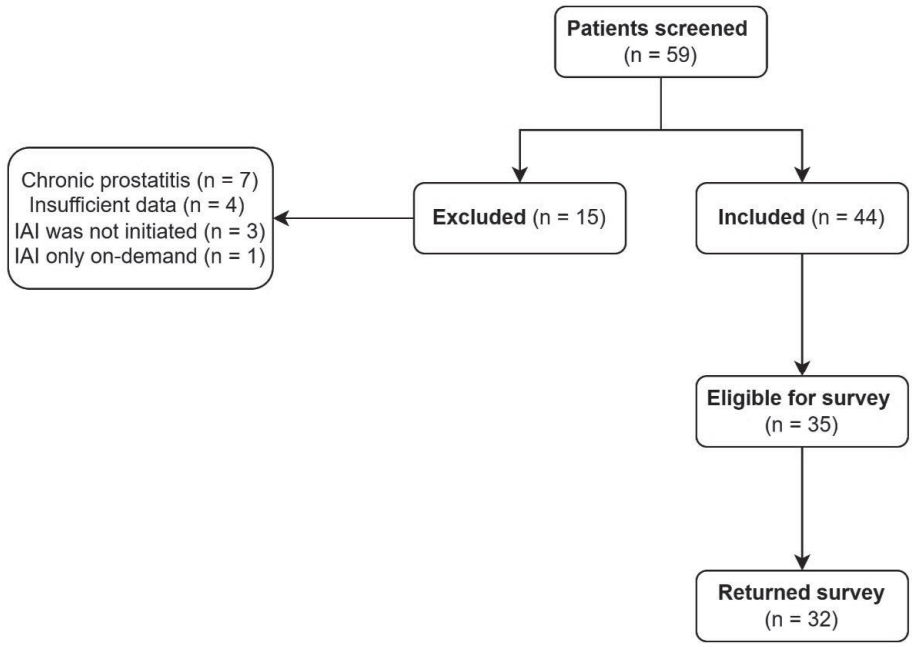
22. Huen KH, Nik-Ahd F, Chen L, Lerman S, Singer J. Neomycin-polymyxin or gentamicin bladder instillations decrease symptomatic urinary tract infections in neurogenic bladder patients on clean intermittent catheterization. J Pediatr Urol 2019; 15(2): 178 e1- e7.

23. Chernyak S, Salamon C. Intravesical Antibiotic Administration in the Treatment of Recurrent Urinary Tract Infections: Promising Results From a Case Series. Female Pelvic Med Reconstr Surg 2020; 26(2): 152-4.

24. Worby CJ, Schreiber HLt, Straub TJ, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol 2022; 7(5): 630-9.

25. Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med 2007; 4(12): e329.

Supplement



Supplementary Figure 1: Flow chart of screening and inclusion process

Supplementary Table 1: Cox regression analysis.

	Hazard ratio (95%CI)	p-value
Cox ME model (no other variables)	0.52 (0.33 – 0.82)	0.005
Cox ME model (including age, gender, and oral prophylaxis)	0.50 (0.31 – 0.79)	0.003
Cox ME model (only first on and off IAI cycle)	0.36 (0.18 – 0.70)	0.003
Cox ME model (extra variable: time between second/third/ fourth IAI cycle and start of first cycle)	0.47 (0.29 – 0.77)	0.002

A mixed-effects model was used to account for multiple (dependent) observations within a patient. Cycle = one 'on' or 'off' treatment period. Abbreviations: ME = mixed effects, IAI = intravesical aminoglycoside instillations

Supplementary Table 2: Subgroup analysis for gender, menopausal status, kidney transplantation and prior CIC.

Subgroup	N	Median time to first recurrence (days)			Number of recurrences on IAI versus off IAI		Interaction term*
		On IAI	Off IAI	p-value	RR (95% CI)	p-value	p-value
Female	31	98	23	0.02	0.59 (0.43 – 0.81)	0.001	0.007
Male	13	114	74	0.90	1.66 (0.87 – 3.18)	0.13	-
Premenopausal	5	89	14	0.06	0.53 (0.25 – 1.11)	0.09	0.85
Postmenopausal	26	98	23	0.04	0.62 (0.44 – 0.87)	0.006	-
Kidney transplant	11	45	82	0.40	1.71 (0.91 – 3.19)	0.10	0.002
Prior CIC	28	104	39.5	0.10	0.82 (0.55 – 1.24)	0.35	0.59

The Kaplan-Meier method was used to estimate time to first recurrence. To compare the incidence of UTI episodes between patients on and off IAI within the stratum, a Poisson mixed effects model was used (with random intercept per patient). * Poisson mixed effects models were made with an interaction term for gender, menopausal status, kidney transplant status and prior CIC status. Menopausal status was evaluated in the stratum of women, all other interaction terms were evaluated in the entire population. Abbreviations: IAI = intravesical aminoglycoside instillations. CIC = clean intermittent catheterisation.

Supplementary Table 3: Sensitivity analysis.

	IAI n (%)	No IAI n (%)	OR (95%CI)	p-value
New-onset LUTS n (%) Not reported = LUTS	160/207 (77.3)	104/116 (89.7)	0.43 (0.18 – 1.06)	0.07
New-onset LUTS n (%) Not reported = no LUTS	122/207 (58.9)	87/116 (75.0)	0.65 (0.32 – 1.32)	0.23
UTI caused by classic gram-negative rods n (%) No culture performed, mixed flora or not reported = gram-negative rods	157/220 (71.4)	96/123(78.0)	0.82 (0.41 – 1.65)	0.58
UTI caused by classic gram-negative rods n (%) No culture performed, mixed flora or not reported = gram-negative rods	101/220 (45.9)	75/123 (61.0)	0.76 (0.41 – 1.42)	0.39
UTI caused by enterococci n (%) No culture performed, mixed flora or not reported = enterococci	82/220 (37.3)	26/123 (21.1)	2.04 (1.11 – 3.75)	0.02
UTI caused by enterococci n (%) No culture performed, mixed flora or not reported = no enterococci	26/220 (11.8)	5/123 (4.1)	3.76 (1.24 – 11.38)	0.02
MDRO/ESBL resistance n (%) No culture performed, mixed flora or not reported = resistance	78/211 (37.0)	39/120 (32.5)	1.06 (0.57 – 1.97)	0.86
MDRO/ESBL resistance n (%) No culture performed, mixed flora or not reported = no resistance	22/211 (10.4)	18/120 (15.0)	0.82 (0.30 – 2.22)	0.69
Hospital admission n (%) Not reported = hospital admission	31/207 (15.0)	10/116 (8.6)	1.13 (0.35 – 3.65)	0.84
Hospital admission n (%) Not reported = no hospital admission	30/207 (14.5)	10/116 (8.6)	1.08 (0.33 – 3.52)	0.90
Number of systemic (oral/intravenous) antibiotics n (%) Not reported = systemic antibiotics	156/207 (75.3)	107/116 (92.2)	0.33 (0.13 – 0.86)	0.02
Number of systemic (oral/intravenous) antibiotics n (%) Not reported = no systemic antibiotics	155/207 (74.9)	107/116 (92.2)	0.32 (0.13 – 0.83)	0.02

Abbreviations: LUTS = lower urinary tract symptoms, UTI = urinary tract infection, MDRO = multidrug resistant organism, ESBL = extended spectrum beta-lactamase, IAI = intravesical aminoglycoside instillations, OR = odds ratio, 95%CI = 95% confidence interval

Chapter 7

Faecal microbiota replacement to eradicate antimicrobial resistant bacteria in the intestinal tract – a systematic review

Manu P. Bilsen, Merel M.C. Lambregts, Joffrey van Prehn, Ed J Kuijper

Curr Opin Gastroenterol. 2022 Jan 1;38(1):15-25

Abstract

Purpose of review

Antimicrobial resistance (AMR) is a rising threat to global health and is associated with increased mortality. Intestinal colonisation with multidrug-resistant organisms (MDRO) can precede invasive infection and facilitates spread within communities and hospitals. Novel decolonisation strategies, such as faecal microbiota transplantation (FMT), are being explored. The purpose of this review is to provide an update on how the field of FMT for MDRO decolonisation has developed during the past year, and to assess the efficacy of FMT for intestinal MDRO decolonisation.

Recent findings

Since 2020, seven highly heterogenous, small, non-randomised cohort studies and five case reports have been published. In line with previous literature, decolonisation rates ranged from 20-90% between studies, and were slightly higher for CRE than VRE. Despite moderate decolonisation rates in two studies, a reduction in MDRO bloodstream and urinary tract infections was observed.

Summary and implications

Although a number of smaller cohort studies show some effect of FMT for MDRO decolonisation, questions remain regarding the true efficacy of FMT (taking spontaneous decolonisation into account), the optimal route of administration, the role of antibiotics pre- and post-FMT and the efficacy in different patient populations. The observed decrease in MDRO infections post-FMT warrants further research.

Introduction

Antimicrobial resistance (AMR) is a rising and significant threat to global health. [1] In addition to the considerable economic burden, AMR is associated with increased morbidity and mortality. [2] In Europe, more than half of *E. coli* isolates are resistant to at least one antimicrobial group and 7.9% of *Klebsiella pneumoniae* isolates are carbapenem resistant. Moreover, there is a worrisome increase in vancomycin-resistant *Enterococcus* (VRE) (18.3%) and infections with extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E). [3, 4] Intestinal colonisation with multidrug-resistant organisms (MDRO) facilitates spread of MDRO within communities and hospitals. In both immunocompetent and immunocompromised hosts, gut colonisation can result in invasive infections, with high morbidity and mortality. [5, 6] In a retrospective, single-centre study including 107 patients undergoing allogeneic stem cell transplantation (allo-SCT), 31% of patients were colonised with at least one MDRO. Compared to non-colonised patients, colonised patients more frequently experienced bacteraemia post-SCT (48% versus 24%) and had a significantly worse two-year overall survival (34% versus 74%), with infection being the leading cause of death. [7]

To prevent infections with MDRO, strategies to combat MDRO colonisation must be explored. The current ESCMID guideline does not recommend the use of non-absorbable antibiotics for MDRO decolonisation, as the available evidence on its efficacy is insufficient. [8] More importantly, non-absorbable antibiotics can contribute to selection of AMR bacteria with subsequent spread to the environment and other individuals. [9]

Faecal microbiota transplantation (FMT) has been shown to be an effective treatment for patients with recurrent *Clostridioides difficile* infection (rCDI), a condition that is characterised by an antibiotic-induced disruption of commensal gut microbiota, i.e. dysbiosis. [10] Compared to healthy stool donors, rCDI patients have decreased microbiota diversity and increased numbers of antibiotic resistant genes. In these patients, FMT increases microbiota diversity, while decreasing the number of antibiotic resistance genes. [11, 12] Contrary to rCDI, less is known about the degree of dysbiosis in individuals with MDRO colonisation, though some studies report decreased species richness in this population as well. [13, 14] Several small studies, including one randomised controlled trial (RCT) [15], have explored whether FMT is an effective modality to decolonise patients with MDRO, as summarised by several recent reviews. [16-18] These reviews conclude that

FMT is a promising treatment strategy for MDRO decolonisation, although the RCT by Huttner et al. [15] did not find a significant difference, but was terminated early. Conclusions are hampered by the major heterogeneity of studies regarding definition of (de)colonisation, type of MDRO, route of administration, number of transplantations, periprocedural treatment with antibiotics, and duration of follow-up.

The objective of this review is to provide an update on how the field of FMT for MDRO decolonisation has developed during the past year, by highlighting recently published and ongoing studies, ultimately to assess whether FMT is an effective treatment strategy for intestinal MDRO decolonisation. Adding to the recent overview provided by Dharmaratne et al. [18], this review includes several newer studies, as well as studies with paediatric patients.

Methods

This systematic review was conducted in accordance with the *Preferred Reporting Items for Systematic reviews and Meta-analyses* (PRISMA) 2020 guidelines. [19] Details of the protocol for this systematic review were registered in PROSPERO. [20]

Eligibility criteria

We included all studies investigating the efficacy of FMT for intestinal MDRO decolonisation. This included clinical trials, cohort studies and case reports in adult and paediatric patients with intestinal MDRO colonisation, including carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem resistant non-fermenters (*Pseudomonas* and *Acinetobacter* spp.), VRE and ESBL-E, confirmed by at least one positive stool sample or rectal/perianal swab. Studies involving immunocompromised patients were eligible for inclusion. We excluded studies only investigating patients colonised with both *Clostridioides difficile* and MDRO, since extreme dysbiosis would be likely in this population. For our intervention (FMT) we considered all routes of administration: oral (capsule), nasogastric/duodenal, via colonoscopy or enema. We applied no restrictions to pretreatment (antibiotics, proton pump inhibitor (PPI) and bowel lavage), stool volume, fresh or frozen stool, donor relationship or number of transplantations. Studies only investigating other microbiota-altering treatments, such as probiotics and non-absorbable antibiotics, were ineligible.

To be included, a study had to report the number of decolonised patients, confirmed by at least one stool sample or rectal/perianal swab post-FMT. Studies reporting the number of MDRO infections post-FMT, e.g. in patients with recurrent urinary tract infections, were only included if they also reported intestinal (de)colonisation. We also included unpublished manuscripts, conference abstracts and ongoing trials. To avoid language bias, studies published in non-English language journals were eligible for inclusion if one of the team members could read the foreign language (French, Spanish, German and Dutch). All study settings (community, outpatient and inpatient) were allowed. We excluded studies published before 2020, since a recent meta-analysis has been performed with studies published before 2020. [18] Finally, we excluded murine (or other animal) studies, reviews and meta-analyses.

Search strategy

Multiple electronic databases were searched May 19th 2021; these included PubMed, Embase, Web of Science, the Cochrane Library, and Academic Search Premier [21]. The search strategy, based on a PICO-style approach, was constructed by librarian specialised in literature searches and is provided in the **Supplement**. Next, a 'snowball' search was performed to identify additional studies by searching reference lists of study reports included in this systematic review or earlier reviews on the same topic. For ongoing trials clinicaltrials.gov was searched July 1st 2021, using the following keywords: 'faecal microbiota transplantation' and 'resistance'. No filters regarding start date were applied, as we did not want to miss ongoing trials that had started before 2020. The entire search was updated in August 2021.

Data extraction and analysis

After removal of duplications in EndNote, references were imported into Covidence software. Title/abstract and full-text screening was performed independently by two reviewers (M.P.B., M.M.C.L.). In case of disagreement, a third researcher was consulted (E.J.K.). A data extraction form was designed, after which one reviewer (M.P.B.) carried out the data extraction using Covidence. For each study, the following data was collected: study design, eligibility criteria, population characteristics, number of participants, type of pathogen, definition of (de) colonisation, detection technique, FMT route of administration, pretreatment, stool volume and type, donor type, decolonisation rate, MDRO infection rate,

microbiota composition and duration of follow-up. The Newcastle Ottawa Scale, addressing three specific domains (i.e. selection, comparability and outcome), was used for assessing risk of bias in cohort studies. [22] Risk of bias was assessed by one reviewer (M.P.B.), but in case of uncertainty, a second reviewer was consulted (M.M.C.L.). A meta-analysis was not undertaken due to significant heterogeneity regarding study design, population and intervention, and a paucity of included studies. A narrative summary of the data is provided below.

Results

Study selection process

The study selection process is summarised in a PRISMA flowchart (**Figure 1**). Most records that were excluded during title and abstract screening involved patients with rCDI. During full-text screening, 35 reports were excluded that either did not include our target population, e.g. patients not colonised with MDRO and receiving FMT for different indications, or did not report intestinal decolonisation rate, e.g. investigating post-FMT faecal composition or decolonisation of extra-intestinal sites instead. Finally, a total of 36 studies were included: seven cohort studies [23-29], five case reports [30-34], and 24 ongoing trials.

Study characteristics

A complete overview of the included cohort studies and case reports is provided in **Table 1**, and ongoing trials are summarised in **Supplementary Table 1**. A total of 254 patients were assessed in the included cohort studies and case reports, with only one study investigating paediatric patients. [28] Eight studies included immunocompromised patients, mostly undergoing allo-SCT [24, 25, 28, 30-34], and three studies included a total of 14 patients with concurrent rCDI. [25, 27, 29] While most studies required one positive stool culture or rectal/perianal swab for the definition of colonisation, decolonisation was often confirmed by serial cultures or swabs. Most patients were colonised with CRE (n = 119), followed by VRE (n = 61), both CRE and VRE (n = 21), ESBL-E (n = 14), and multidrug resistant *Pseudomonas aeruginosa* (n = 1). Ghani et al. [25]* did not specify the type of MDRO for their control group. To the best of our knowledge, the study by Wang et al. [34] is the first study investigating the efficacy of FMT for gut eradication of a hypervirulent *Klebsiella pneumoniae* strain.

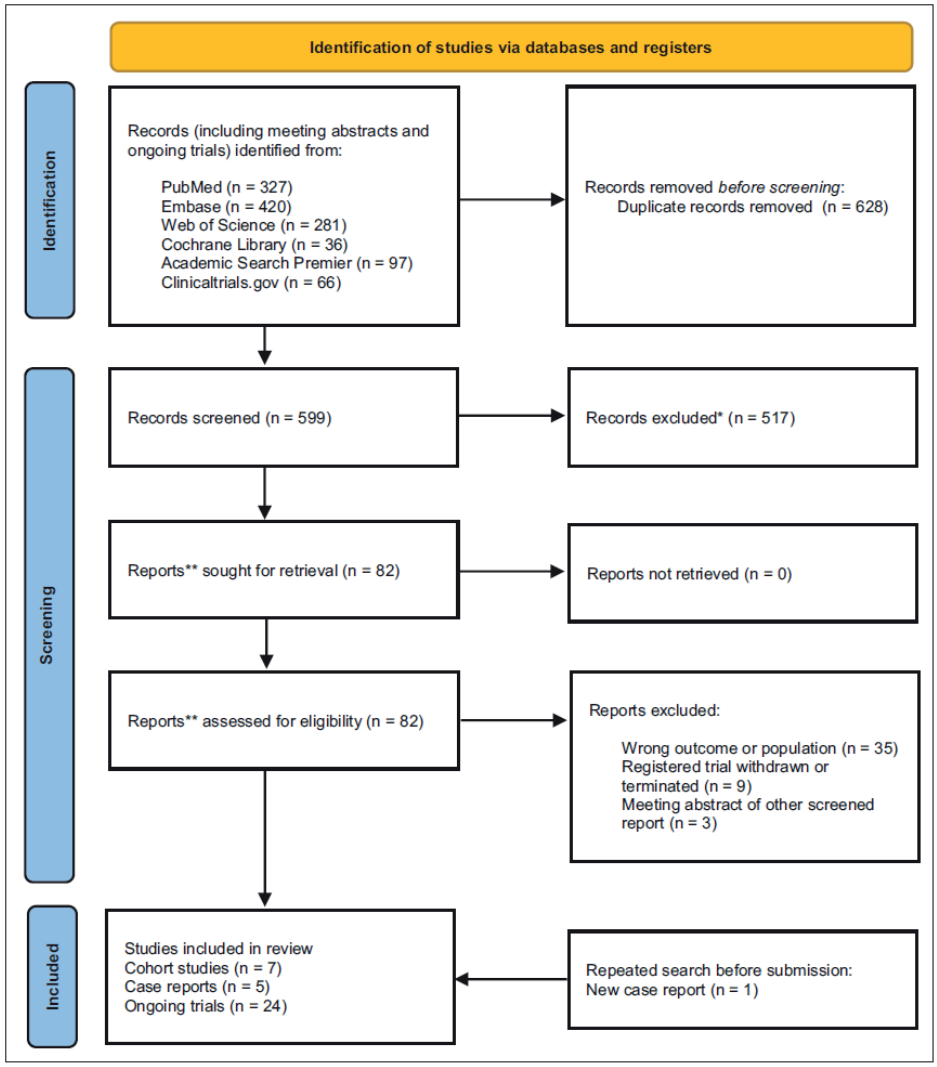


Figure 1: PRISMA flowchart of study selection process. *Large number of records involved patients with recurrent *C. difficile*. **In case of ongoing trials, we assessed the study protocol for eligibility.

Table 1: Overview of included cohort studies and case reports.

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Lee 2020 Korea (23)	Prospective cohort study with control group	Adult patients with CRE or VRE colonisation Median age, gender, immune status not reported	Total (n = 38) FMT (n = 21) Control (n = 17)	Colonisation: 2 Decolonisation: NR	FMT cohort: CRE (n = 13, not further specified), VRE (n = 5), CRE and VRE (n = 3) Controls: CRE (n = 7), VRE (n = 10)	NR	NR	NR	FMT: 8/20 (40%) at 1 month, 10/14 (71.4%) at 3 months Controls: 0/14 (0%) at 1 month, 1/9 (11.1%) at 3 months	NR
Bar-Yoseph 2020 Israel (24)	Prospective cohort study with control group	Adult patients with CRE colonisation Median age: 62 years Male gender: 53% Immunocompro-mised: 20.5%	Total (n = 39) FMT (n = 15) Control (n = 24)	Colonisation: 1 Decolonisation: 3	FMT cohort: *Klebsiella pneumoniae* (n = 7), *Enterobacter* spp. (n = 3), *E. coli* (n = 2), *Serratia marcescens* (n = 2), *Klebsiella oxytoca* (n = 1) Controls: *Klebsiella pneumoniae* (n = 19), *Enterobacter* spp. (n = 2), *E. coli* (n = 3), *Citrobacter freundii* (n = 1)	Oral (15 capsules per day for 2 consecutive days) Stool: 25-30 gram, frozen, unrelated donor	AB: no BL: no PPI: yes	FMT cohort: 5/15 (33.3%) Controls: 21/24 (87.5%)	FMT: 9/15 (60%) at 1 month, 8/12 (66.7%) at 6 months Controls: 10/24 (41.7%) at 1 month, 7/13 (53.8%) at 6 months	FMT: 0/15 Controls: 9/24 (37.5%)

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Ghani 2020 United Kingdom (25)	Prospective cohort study with control group	**Group 1:** Haematology patients (mostly allo-HSCT) with CRE, VRE or ESBL colonisation **Group 2:** Patients with MDRO-mediated rUTI, mostly renal transplant recipients, no current infection **Controls:** similar patients but not undergoing FMT Median age 62.5 years Male gender 55% Immunocompromised: 76% rCDI (n = 4)	Total (n = 60) Group 1 (n = 11) Group 2 (n = 9) Control (n = 40)	NR (just 'serial rectal swabs')	Group 1: CRE (n = 8, including *E. coli*, *Citrobacter freundii* and *Klebsiella* spp.) VRE (n = 3) or ESBL *E. coli* (n = 2) Group 2: ESBL (n = 9): *E. coli* (n = 7), *Klebsiella pneumoniae* (n = 2)	Upper endoscopy/ naso-duodenal tube Stool: 50 gram, frozen, unrelated donor, 1–2 FMTs per patient	AB: discontinued 24h prior BL: yes PPI: yes	Yes, almost all patients, no absolute number (or specific antibiotic) is reported	7/17 (41%) of group 1 and 2 patients were decolonised (follow-up range 6 weeks - 24 months), NR for control group	Significant reduction in BSI (absolute number NR) and MDRO UTIs (pre-FMT median = 4 ± 2 episodes, post-FMT median = 1 ± 2 episodes) in group 1 and 2 compared to controls.
Seong 2020 Korea (26)	Prospective cohort study with control group	Adult patients with CRE or VRE colonisation Median age: 69 years Male gender: 53% Immunocompromised: none	Total (n = 83) FMT (n = 35) Control (n = 48)	Colonisation: 1 Decolonisation: 2	FMT cohort: VRE 19/35 (54.3%), CRE 4/35 (11.4%), both 12/35 (34.3%) Controls: VRE 24/48 (50%), CRE 20/48 (41.7%), both 4/48 (8.3%)	At the discretion of the physician: upper endoscopy, oral or colonoscopy Stool: 100 gram, frozen, unrelated donor, 1 FMT per patient	AB: 45% in the week prior BL: yes if colonoscopy PPI: yes if upper endoscopy	19/35 (54.3%) in the week post-FMT	FMT: 65.7% at 6 months, 68.6% at 12 months Controls: 25.0% at 6 months, 27.1% at 12 months	NR

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Lee 2021 Korea (27)	Prospective cohort study without control group	Adult patients with CRE or VRE colonisation Median age: 75 years Male gender: 30% Immunocompromised: NR rCDI (n = 2)	N = 10	Colonisation: NR Decolonisation: 3	*Klebsiella pneumoniae*, carbapenemase producing (n = 8), VRE and *Klebsiella pneumoniae* (n = 2)	Colonoscopy (n = 9), upper endoscopy (n = 7), 20 capsules (n = 1) Stool: volume NR, frozen, unrelated donor, 1–3 FMTs per patient	AB: discontinued 48h prior BL: yes PPI: no	NR	4/10 at 1 month, 5/10 at 3 months and 9/10 at 5 months after initial FMT	NR
Merli 2020 Italy (28)	Prospective cohort study without control group	Paediatric patients scheduled to undergo allo-HSCT, some having a history of systemic infections with MDRO Median age: 11 years Male gender 80% Immunocompromised: 100%	N = 5	NR (just 'weekly rectal swabs)	Carbapenemase resistant: *E. coli* (n = 3), *Klebsiella pneumoniae* (n = 2), *Klebsiella oxytoca* (n = 1), *Klebsiella ornithinolytica* (n = 1), *Enterobacter cloacae* (n = 1), *Pseudomonas aeruginosa* (n = 1)	Upper endoscopy/ naso-duodenal tube Stool: 100–240 mL, frozen (80%), unrelated donor, 1 FMT per patient	AB: yes, 80% received oral colistin for 3 days BL: no PPI: no	Yes, broad-spectrum antibiotic prophylaxis with piperacillin/ tazobactam when neutrophils <500/µl or fever	4/5 (80%) at 1 week, 1/5 (20%) at 1 month	1 episode in 1 patient

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Silva 2020 Portugal (29)	Retrospective cohort study	Adult patients with CRE colonisation Median age: 66 years Male gender: 38.4% Immunocompromised: none rCDI (n = 8)	N = 13	Colonisation: 1 Decolonisation: 3	CRE, not further specified	Upper endoscopy/ naso-duodenal tube Stool: 50 mL, fresh, unrelated donor, number of FMTs NR	AB: only for rCDI patients (until the day before FMT) BL: yes PPI: yes	No	Total: 10/13 (77%) Without rCDI (CRE carriers only): 4/5 (80%), median time to decolonisation 16 weeks	0

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Biernat 2020 Poland (30)	Case report	Both patients underwent allo-HSCT (one for AML, one for osteomyelofibrosis) Median age: 28.5 years Male (both patients) Immunocompromised: 100%	N = 2	Colonisation: 1 Decolonisation: 1	Case 1: ESBL *E. coli* and ESBL *Klebsiella pneumoniae* Case 2: ESBL *Enterobacter cloacae*	Upper endoscopy/ naso-duodenal tube Stool: 100 gram, fresh, unrelated donor, 3-4 FMTs per patient	AB: stopped prior to FMT (but recent broad spectrum treatment) BL: no PPI: yes	Yes	Case 1: Eradication of ESBL *E. coli* after first FMT and eradication of ESBL *Klebsiella* after third FMT. Acquired VRE after second FMT, eradicated after third. Colonised with MDR *Acinetobacter baumannii* after third FMT Case 2: Eradication of ESBL *E. cloacae* after first FMT, acquired VRE and ESBL *E. coli* after second and third FMT, eradicated after fourth FMT	1/2 Case 1 died due to *Acinetobacter. baumannii* BSI

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Bilinski 2020 Poland (31)	Case report	Adult with AML undergoing allo-HSCT Age: 36 years Male Immunocompromised: yes	N = 1	Colonisation: 1 Decolonisation: 3	CRE (*Klebsiella pneumoniae*, NDM-1)	Upper endoscopy/ naso-duodenal tube Stool: 100 gram, fresh, unrelated donor, 2 FMTs	AB: no BL: yes PPI: yes	Yes, metronidazole after first FMT	1/1 at 2 weeks but reappeared after chemotherapy and antibiotic prophylaxis. After a second FMT the patient remained decolonised at 6 months	0
Keen 2020 United States (32)	Case report	Patient with rUTI due to ESBL *Klebsiella pneumoniae*. History of kidney and liver transplantation Age: 62 years Female Immunocompromised: yes	N = 1	Colonisation: 1 Decolonisation: NR (but patient was tested multiple times)	ESBL *Klebsiella pneumoniae*	Enema Stool: single 150 mL suspension (> 10^7 organisms per mL), frozen, unrelated donor, 1 FMT	AB: suppressive ertapenem until 2 days prior to FMT BL: no PPI: no	Yes, oral amoxicillin 6 weeks post-FMT, then intravenous vancomycin, piperacillin/ tazobactam 8 weeks post-FMT and amoxicillin/ clavulanate, followed by cefepime and metronidazole 10 weeks post-FMT	0/1 at 1 month and 4 months post-FMT	2

Table 1: Continued

First author & year	Study design	Population	Number of participants	Number of culture/PCR to define (de) colonisation	Type of pathogen*	FMT procedure**	Pretreatment	Antibiotic use post-FMT	Decolonisation rate	Number of MDRO infections post-FMT
Su 2021 China (33)	Case report	Patient with AML undergoing allo-HSCT, colonised with CRE prior to conditioning therapy, identified on routine rectal screening. Age: 45 years Male Immunocompromised: yes	N = 1	Colonisation: 1 Decolonisation: NR (but patient was tested seven times)	Carbapenem resistant *Klebsiella pneumoniae*	Upper endoscopy/ naso-duodenal tube Stool: volume NR, frozen, unrelated donor, 2 courses with 17 day interval (three procedures per course)	AB: no BL: no PPI: no	No	1/1 (stool cultures were CRE negative at 1 week, 1 month, 2 months, 3 months, 6 months, 11 months, and 26 months)	0
Wang 2021 (34)	Case report	Renal transplant patient with CRE bacteraemia and surgical site infection Age: 37 years Female Immunocompromised: yes	N = 1	Colonisation: 2 Decolonisation: 1	Carbapenem resistant and hypervirulent *Klebsiella pneumoniae*	Upper endoscopy/ naso-duodenal tube Stool: volume NR, fresh/ frozen NR, unrelated donor, 1 FMT	AB: meropenem, tigecycline, fosfomycin discontinued 24h prior to FMT BL: yes PPI: yes	No	1/1 at 1 week	0

*May surpass total number of patients as some patients were colonised with multiple MDROs. ** May surpass total number of patients as some patients had multiple FMTs with different procedures. Abbreviations: CRE = carbapenemase resistant Enterobacteriaceae, VRE = vancomycin resistant *Enterococcus*, allo-HSCT = allogeneic haematopoietic stem cell transplantation, ESBL = extended spectrum beta-lactamase, MDRO = multidrug resistant organism, rUTI = recurrent urinary tract infection, FMT = faecal microbiota transplantation, rCDI = recurrent *Clostridioides difficile* infection, AML = acute myeloid leukaemia, NR = not reported, NDM-1 = New Delhi Metallo-beta-lactamase – 1, AB = antibiotics, BL = bowel lavage, PPI = proton pump inhibitor, BSI = bloodstream infection

FMT procedure

The primary route of administration for FMT was upper endoscopy; a minority of studies used capsules, enemas or colonoscopy. Whereas stool volume varied (from 25-100 gram), all stool samples were obtained from healthy, unrelated donors, and were mostly frozen. One study [28] pretreated patients with non-absorbable antibiotics (oral colistin), and in seven studies patients had used antibiotics in the week prior to FMT. [25-27, 29, 30, 32, 34] Patients were pretreated with PPI in seven studies, and bowel lavage in six studies. Moreover, the number of transplantations varied, with six studies performing multiple transplantations per patient.

FMT efficacy: decolonisation and infection rate

In the seven included cohort studies investigating any MDRO, decolonisation rates ranged from 20-90% for patients treated with FMT and 11-66% for controls. Duration of follow-up varied from 1-24 months. The largest between group difference was seen in the prospective cohort study by Lee et al. [23], i.e. a decolonisation rate of 71.4% versus 11.1% for FMT patients and controls respectively. Of note, duration of follow-up was only 3 months, while spontaneous decolonisation usually occurs at a later time point. [9] In the largest study performed thus far [26]**, decolonisation rates were 65.7% (FMT) versus 25.0% (controls) at 6 months, and remained similar at 12 months (68.6% versus 27.1% for FMT patients and controls respectively).

Four of seven cohort studies included both CRE and VRE patients. Of these, two reported decolonisation rates for CRE and VRE patients separately. [23, 26] In the study by Lee et al. [23] CRE decolonisation rate at 3 months was 88.9% (8/9 patients) for the FMT group and 25% (1/4 patients) for the control group. For VRE patients, decolonisation was only reported for 1 month post-FMT, being 60% (3/5 patients) for the FMT group and 0% (number of patients not specified) for the control group. In the study by Seong et al. [26]**, the 12-month decolonisation rate for CRE patients was 75% (3/4 patients) and 45% (9/20 patients) for the FMT and control group respectively. For VRE patients, a 12-month decolonisation rate of 52.6% (10/19 patients) for the FMT group and 12.5% (3/12 patients) for the control group was observed.

In the study by Merli et al. [28] decolonisation was achieved for four out of five paediatric recipients after 1 week, but all four patients were recolonised after 1

month. All patients received antibiotic prophylaxis after a minimum of 3 days post-FMT, as part of the conditioning regimen for allo-SCT. Recolonisation also occurred during antibiotic prophylaxis (for allo-SCT) in an adult patient. [31] Silva et al. [29], Su et al. [33] and Wang et al. [34] were the only studies in which patients did not receive antibiotics after FMT. Prolonged decolonisation was achieved in four out of five CRE patients in the first study, and in both patients in the case reports.

The occurrence of MDRO infections was reported in four out of seven cohort studies. In the two studies with a control group [24, 25], MDRO infections were less frequent in the intervention group. While Bar-Yoseph et al. [24] showed a modest decolonisation rate 6 months post-FMT (66.7%), no MDRO infections occurred in the FMT group. In contrast, 37.5% of patients in the control group experienced MDRO infections. A similar effect was reported by Ghani et al. [25], where only 41% of patients achieved decolonisation, but there was a significant reduction in bloodstream infections (BSI) (no haematology patient developed bacteraemia with their pre-FMT MDRO) and MDRO UTIs (pre-FMT median = 4 ± 2 episodes, post-FMT median = 1 ± 2 episodes), compared to controls.

Microbiota composition pre- and post-FMT

Three case reports [32-34] and two cohort studies [26, 28] reported pre-FMT microbiota composition of patients with MDRO colonisation. Dysbiosis was seen in all patients of the case reports, with Proteobacteria making up more than a third of their gut microbiota, most likely due to prolonged broad-spectrum antimicrobial therapy prior to FMT. Low species richness was also seen in several patients in the study by Merli et al. [28], with one patient having a microbiota profile that was almost exclusively comprised of Enterobacteriaceae (97%). Moreover, Seong et al. [26] showed that patients colonised with VRE had higher counts of Proteobacteria en Verrucomicrobia than healthy stool donors. Seven studies reported faecal microbiota composition after FMT. [24, 26-28, 32-34] Bar-Yoseph [24] showed that post-FMT stool samples of responders, i.e. successfully decolonised patients, resembled those of donors, which was not seen for non-responders. While abundance of Enterobacteriaceae decreased in post-FMT stool samples of responders, it increased for non-responders. After FMT, significantly higher counts of *Bifidobacterium bifidum* were observed in samples of responders, compared to non-responders. Lee et al. [27] showed greater microbiota diversity

post-FMT, with a significantly increased abundance of Bacteroidetes, which was also observed in three case reports. [32-34]

Ongoing trials

Currently, there are 24 ongoing trials investigating FMT for MDRO decolonisation, including 13 RCTs and 11 prospective cohort studies. The largest RCT (NCT04431934) is aiming to enrol 437 patients and is expected to be completed December 2022. Very few studies have posted preliminary results, as shown in **Supplementary Table 1**.

Risk of bias assessment

A summary of the risk of bias assessments for the included cohort studies is presented in **Supplementary Table 2**. Overall, there were concerns about risk of bias for two out of seven cohort studies [23, 25], mainly due to dropouts (without description of those lost), and inadequate descriptions of the study population and outcomes.

Discussion

In this narrative review, we provide an overview of recent studies investigating the efficacy of FMT for MDRO decolonisation. Only a few studies have addressed this question since 2020. In line with earlier reviews on the same topic [16, 17, 35, 36], decolonisation rates varied greatly. Although only two studies reported decolonisation rates for CRE and VRE separately and sample sizes were small, decolonisation rates were higher for CRE patients, with a large effect size compared to controls. To date, only one RCT investigating the efficacy of FMT for MDRO decolonisation has been published. [15] In this study, 39 immunocompetent ESBL-E or CRE carriers were randomised to either no intervention or a 5 day course of oral colistin and neomycin followed by FMT. After 35-48 days, there was no significant difference regarding decolonisation rate between the two groups (41% versus 29% for FMT patients and controls respectively). However, the study was limited by not reaching the calculated sample size, using different routes of administration (nasogastric tube and capsules) and pretreating patients with antibiotics in the intervention arm. Furthermore, control subjects were not treated with antibiotics, further complicating assessment of the true efficacy of FMT.

A previous review by Yoon et al. [16] showed that post-FMT antibiotic use led to lower decolonisation rates. While we could not draw any firm conclusions from our included studies, we did observe that recolonisation and a high number of MDRO infections occurred in patients that had received antibiotics post-FMT. This could be explained by the finding that post-FMT antibiotic use can blunt FMT engraftment, as shown by metagenomic analysis in another study. [24]* Another phenomenon that needs to be taken into consideration when interpreting results is spontaneous decolonisation. A systematic review and meta-analysis by Bar-Yoseph et al. [9] showed that, in health care settings, ESBL-E and CRE colonisation rates spontaneously decreased from 80.2% and 73.9% at 1 month to 35.7% and 34.6% at 12 months respectively. In another systematic review including thirteen studies (n = 1936 patients) 80% of VRE patients were decolonised after 40 weeks, however not all studies confirmed decolonisation with three separate swabs. [37] These findings raise the possibility that decolonisation may be falsely attributed to FMT and underline the necessity of a control group when trying to establish the true efficacy of FMT for MDRO decolonisation. Despite this fact, only four of our included studies had a control group, considerably limiting the evidence included in our review. Notably, only two other controlled studies have been conducted prior to 2020. [15, 38]

Intriguingly, while decolonisation rates in two of the larger included cohort studies were moderate, a major reduction in MDRO infections was observed. [24, 25] In another prospective cohort study assessing the incidence of BSI in rCDI patients treated with either FMT or antibiotics, FMT patients had significantly fewer BSI than patients treated with antibiotics (4% versus 26%). [39] The authors hypothesise that FMT may have aided in increasing colonisation resistance by restoring a disturbed microbiota. This may be accompanied by decreasing intestinal permeability (by treating CDI) and thus preventing translocation of Gram negative bacteria into the bloodstream. Other possible explanations include that FMT can reduce inflammation (and thereby translocation) as is observed in patients with inflammatory bowel disease or graft-versus-host disease, similar to patients in the study by Ghani et al. [25, 40, 41] Lastly, even though FMT might not have eradicated the MDRO from the gut completely, it may have reduced the abundance of *Enterobacteriaceae*, and thereby reduced the likelihood of BSI.

Next to the low number of controlled studies, the evidence included in our review is limited by small samples sizes. Two studies reported dropouts, but did not provide a description of those lost. In addition, most studies defined colonisation

as one positive stool culture (or PCR) or rectal/perianal swab, while colonisation is usually defined as at least two consecutive (positive) samples with the most recent confirmation one week prior to FMT. We chose not to exclude studies that only used one culture or PCR to define colonisation, since this would have significantly reduced the number of eligible studies. Moreover, we observed considerable heterogeneity between studies regarding study population (e.g. including immunocompromised patients), type of pathogens, FMT procedure and post-FMT antibiotic use. Therefore, we need to exercise caution in interpreting the results mentioned in **Table 1**. Since eight studies included immunocompromised patients, one might question the generalisability of the results. Although based on small numbers, the systematic review by Yoon et al. [16] showed higher decolonisation rates for immunocompromised patients, compared to immunocompetent patients. For rCDI, FMT is as effective in immunocompromised patients as in immunocompetent patients. [42] Nevertheless, invasive MDRO infections are a considerable problem in immunocompromised patients, highlighting the importance of researching the role of FMT in this specific population.

Our review process had some methodological limitations. While title/abstract and full-text screening was done by two reviewers independently, data extraction and risk of bias assessment was done by one reviewer. However, a second reviewer was always consulted in case of doubt. In case of missing data, we did not contact study authors. Strengths of our review include our comprehensive search strategy, including many databases, searching for meeting abstracts, and repeating the search before submission of our manuscript.

Future research should include sufficiently powered RCTs with an adequate duration of follow-up to account for spontaneous decolonisation. The protocol for FMT should be standardised with one or more treatments, including the use of different donors to study donor effects. It is possible that different strategies should be applied to CRE and VRE gut eradication. Moreover, more stringent definitions of (de)colonisation should be applied and different pre- and post-treatments and routes of administration should be compared to optimise efficacy. Next to decolonisation, the number of MDRO infections post-FMT should be assessed. As shown in **Supplementary Table 1**, several large RCTs, including both immunocompromised and immunocompetent patients, are currently recruiting. At least one RCT (NCT04188743) is using a more stringent definition of colonisation, requiring at least two positive rectal swabs prior to FMT. The same RCT is comparing the efficacy of donor stool to autologous FMT. Another RCT

(NCT04181112) is pretreating one group with antibiotics, while not pretreating the other group. Different routes of administration are being investigated, though they are not being compared head-to-head within a single upcoming trial.

Conclusion

Since 2020, only a handful of smaller, non-controlled studies investigating the efficacy of FMT for MDRO decolonisation have been published. Although a number of these cohort studies show some effect of FMT for MDRO decolonisation, questions remain regarding the true efficacy of FMT (taking spontaneous decolonisation into account), the optimal route of administration, the role of pre- and post-FMT antibiotic use, and the efficacy in different patient populations. Interestingly, despite modest decolonisation rates, FMT reduced the number of MDRO infections, a finding warranting further exploration.

Acknowledgements

The authors would like to thank J.W. Schoones for his excellent help with constructing our search strategy.

References

1. Global antimicrobial resistance surveillance system (GLASS) report: early implementation. Available at: https://apps.who.int/iris/handle/10665/332081.
2. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC. **2019**.
3. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. Stockholm: ECDC. **2020**.
4. Jernigan JA, Hatfield KM, Wolford H, et al. Multidrug-Resistant Bacterial Infections in U.S. Hospitalized Patients, 2012-2017. N Engl J Med **2020**; 382(14): 1309-19.
5. Pop-Vicas A, Mitchell SL, Kandel R, Schreiber R, D'Agata EM. Multidrug-resistant gram-negative bacteria in a long-term care facility: prevalence and risk factors. J Am Geriatr Soc **2008**; 56(7): 1276-80.
6. Pop-Vicas AE, D'Agata EM. The rising influx of multidrug-resistant gram-negative bacilli into a tertiary care hospital. Clin Infect Dis **2005**; 40(12): 1792-8.
7. Bilinski J, Robak K, Peric Z, et al. Impact of Gut Colonization by Antibiotic-Resistant Bacteria on the Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective, Single-Center Study. Biol Blood Marrow Transplant **2016**; 22(6): 1087-93.
8. Tacconelli E, Mazzaferri F, de Smet AM, et al. ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria carriers. Clin Microbiol Infect **2019**; 25(7): 807-17.
9. Bar-Yoseph H, Hussein K, Braun E, Paul M. Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. J Antimicrob Chemother **2016**; 71(10): 2729-39.
10. Terveer EM, Vendrik KE, Ooijevaar RE, et al. Faecal microbiota transplantation for Clostridioides difficile infection: Four years' experience of the Netherlands Donor Feces Bank. United European Gastroenterol J **2020**; 8(10): 1236-47.
11. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. Clin Infect Dis **2016**; 62(12): 1479-86.
12. Song Y, Garg S, Girotra M, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. PLoS One **2013**; 8(11): e81330.
13. Korach-Rechtman H, Hreish M, Fried C, et al. Intestinal Dysbiosis in Carriers of Carbapenem-Resistant Enterobacteriaceae. mSphere **2020**; 5(2).
14. Araos R, Montgomery V, Ugalde JA, Snyder GM, D'Agata EMC. Microbial Disruption Indices to Detect Colonization With Multidrug-Resistant Organisms. Infect Control Hosp Epidemiol **2017**; 38(11): 1312-8.
15. Huttner BD, de Lastours V, Wassenberg M, et al. A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. Clin Microbiol Infect **2019**; 25(7): 830-8.

16. Yoon YK, Suh JW, Kang EJ, Kim JY. Efficacy and safety of fecal microbiota transplantation for decolonization of intestinal multidrug-resistant microorganism carriage: beyond Clostridioides difficile infection. Ann Med **2019**; 51(7-8): 379-89.

17. Saha S, Tariq R, Tosh PK, Pardi DS, Khanna S. Faecal microbiota transplantation for eradicating carriage of multidrug-resistant organisms: a systematic review. Clin Microbiol Infect **2019**; 25(8): 958-63.

18. Dharmaratne P, Rahman N, Leung A, Ip M. Is there a role of faecal microbiota transplantation in reducing antibiotic resistance burden in gut? A systematic review and Meta-analysis. Ann Med **2021**; 53(1): 662-81.

19. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ **2021**; 372: n71.

20. https://www.crd.york.ac.uk/prospero Record ID 259623.

21. https://www.ebsco.com/products/research-databases/academic-search-premier.

22. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses.

23. Lee JH, Shin JB, Ko WJ, Kwon KS, Kim H, Shin YW. Efficacy and safety of fecal microbiota transplantation on clearance of multi-drug resistance organism in multicomorbid patients: A prospective non-randomized comparison trial. United European Gastroenterology Journal **2020**; 8 (8 SUPPL): 499.

24. Bar-Yoseph H, Carasso S, Shklar S, et al. Oral capsulized Fecal microbiota transplantation for eradication of carbapenemase-producing Enterobacteriaceae colonization with a metagenomic perspective. Clin Infect Dis **2020**.

25. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. Clin Infect Dis **2021**; 72(8): 1444-7.

26. Seong H, Lee SK, Cheon JH, et al. Fecal Microbiota Transplantation for multidrug-resistant organism: Efficacy and Response prediction. J Infect **2020**; 81(5): 719-25.

27. Lee JJ, Yong D, Suk KT, et al. Alteration of Gut Microbiota in Carbapenem-Resistant Enterobacteriaceae Carriers during Fecal Microbiota Transplantation According to Decolonization Periods. Microorganisms **2021**; 9(2).

28. Merli P, Putignani L, Ruggeri A, et al. Decolonization of multi-drug resistant bacteria by fecal microbiota transplantation in five pediatric patients before allogeneic hematopoietic stem cell transplantation: gut microbiota profiling, infectious and clinical outcomes. Haematologica **2020**; 105(11): 2686-90.

29. Silva JC, Ponte A, Mota M, et al. Fecal microbiota transplantation in the intestinal decolonization of carbapenamase-producing enterobacteriaceae. Rev Esp Enferm Dig **2020**; 112(12): 925-8.

30. Biernat MM, Urbaniak-Kujda D, Dybko J, Kapelko-Słowik K, Prajs I, Wróbel T. Fecal microbiota transplantation in the treatment of intestinal steroid-resistant graft-versus-host disease: two case reports and a review of the literature. J Int Med Res **2020**; 48(6): 300060520925693.

31. Bilinski J, Lis K, Tomaszewska A, et al. Eosinophilic gastroenteritis and graft-versus-host disease induced by transmission of Norovirus with fecal microbiota transplant. Transpl Infect Dis **2021**; 23(1): e13386.

32. Keen EC, Tasoff P, Hink T, et al. Microbiome Restoration by RBX2660 Does Not Preclude Recurrence of Multidrug-Resistant Urinary Tract Infection Following Subsequent Antibiotic Exposure: A Case Report. Open Forum Infect Dis **2020**; 7(3): ofaa042.

33. Su F, Luo Y, Yu J, et al. Tandem fecal microbiota transplantation cycles in an allogeneic hematopoietic stem cell transplant recipient targeting carbapenem-resistant Enterobacteriaceae colonization: a case report and literature review. Eur J Med Res **2021**; 26(1): 37.

34. Wang J, Li X, Wu X, et al. Fecal Microbiota Transplantation as an Effective Treatment for Carbapenem-Resistant Klebsiella pneumoniae Infection in a Renal Transplant Patient. Infect Drug Resist **2021**; 14: 1805-11.

35. Feehan A, Garcia-Diaz J. Bacterial, Gut Microbiome-Modifying Therapies to Defend against Multidrug Resistant Organisms. Microorganisms **2020**; 8(2).

36. Amrane S, Lagier JC. Faecal Microbiota Transplantation for antibiotic resistant bacteria decolonization. Human Microbiome Journal **2020**; 16.

37. Shenoy ES, Paras ML, Noubary F, Walensky RP, Hooper DC. Natural history of colonization with methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE): a systematic review. BMC Infect Dis **2014**; 14: 177.

38. Saidani N, Lagier JC, Cassir N, et al. Faecal microbiota transplantation shortens the colonisation period and allows re-entry of patients carrying carbapenamase-producing bacteria into medical care facilities. Int J Antimicrob Agents **2019**; 53(4): 355-61.

39. Ianiro G, Murri R, Sciume GD, et al. Incidence of Bloodstream Infections, Length of Hospital Stay, and Survival in Patients With Recurrent Clostridioides difficile Infection Treated With Fecal Microbiota Transplantation or Antibiotics: A Prospective Cohort Study. Ann Intern Med **2019**; 171(10): 695-702.

40. Quraishi MN, Shaheen W, Oo YH, Iqbal TH. Immunological mechanisms underpinning faecal microbiota transplantation for the treatment of inflammatory bowel disease. Clin Exp Immunol **2020**; 199(1): 24-38.

41. van Lier YF, Davids M, Haverkate NJE, et al. Donor fecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. Sci Transl Med **2020**; 12(556).

42. Shogbesan O, Poudel DR, Victor S, et al. A Systematic Review of the Efficacy and Safety of Fecal Microbiota Transplant for Clostridium difficile Infection in Immunocompromised Patients. Can J Gastroenterol Hepatol **2018**; 2018: 1394379.

Supplement
Search strategy

Databases:

PubMed

http://www.ncbi.nlm.nih.gov/pubmed?otool=leiden

((("Fecal Microbiota Transplantation"[Mesh] OR "fecal microbiota transplantation"[tw] OR "fecal microbiota transplantations"[tw] OR "fecal microbiota transplant"[tw] OR "fecal microbiota transplant*"[tw] OR "fecal microbiota transfer"[tw] OR "fecal microbiota transfer*"[tw] OR "faecal microbiota transplantation"[tw] OR "faecal microbiota transplant"[tw] OR "faecal microbiota transplant*"[tw] OR "faecal microbiota transfer"[tw] OR "faecal microbiota transfer*"[tw] OR "Intestinal Microbiota Transfer"[tw] OR "Intestinal Microbiota Transplantation"[tw] OR "Intestinal Microbiota transplant"[tw] OR "Intestinal Microbiota transplant*"[tw] OR "Donor Feces Infusion"[tw] OR "Donor Feces"[tw] OR "Donor Faeces"[tw] OR "Donor Fecal"[tw] OR "Donor Faecal"[tw] OR "fecal microbial transplantation"[tw] OR "fecal microbial transplant"[tw] OR "fecal microbial transplant*"[tw] OR "fecal microbial transfer"[tw] OR "fecal microbial transfer*"[tw] OR "faecal microbial transplantation"[tw] OR "faecal microbial transplant"[tw] OR "faecal microbial transplant*"[tw] OR "fecal transplantation"[tw] OR "fecal transplant"[tw] OR "fecal transplant*"[tw] OR "fecal transfer"[tw] OR "fecal transfer*"[tw] OR "faecal transplantation"[tw] OR "faecal transplant"[tw] OR "faecal transplant*"[tw] OR "fecal microbiome transplantation"[tw] OR "fecal microbiome transplantations"[tw] OR "fecal microbiome transplant"[tw] OR "fecal microbiome transplant*"[tw] OR "fecal microbiome transfer"[tw] OR "fecal microbiome transfer*"[tw] OR "faecal microbiome transplantation"[tw] OR "faecal microbiome transplant*"[tw] OR (("fecal microbiota"[tw] OR "feces microbiota"[tw] OR "faecal microbiota"[tw] OR "faeces microbiota"[tw] OR "fecal microb*"[tw] OR "feces microb*"[tw] OR "faecal microb*"[tw] OR "faeces microb*"[tw]) AND ("transplant*"[tw]))) AND ("colonization"[tw] OR "colonisation"[tw] OR "decolonization"[tw] OR "decolonisation"[tw] OR "coloniz*"[tw] OR "colonis*"[tw] OR "decoloniz*"[tw] OR "decolonis*"[tw] OR "antibiotic resistance"[tw] OR "Drug Resistance, Microbial"[Mesh] OR "multi-drug resistant"[tw] OR "multi-drug resistance"[tw] OR "multidrug resistant"[tw] OR "multidrug resistance"[tw] OR "carbapenem"[tw] OR "Carbapenems"[Mesh] OR "Carbapenem*"[tw] OR "Doripenem"[tw] OR "Ertapenem"[tw] OR "Thienamycins"[tw] OR "Thienamycin"[tw] OR "Imipenem"[tw] OR "Meropenem"[tw] OR "vancomycin"[tw] OR "Vancomycin"[Mesh] OR "Vancomycin*"[tw] OR "ESBL"[tw] OR "extended spectrum"[tw] OR "extendedspectrum"[tw] OR "extended spectr*"[tw] OR "extendedspectr*"[tw] OR "multi-resistant"[tw] OR "drug resistant"[tw] OR "multi-resistance"[tw] OR "drug resistance"[tw] OR "multi-resistan*"[tw] OR "drug resistan*"[tw]) AND ("2019/01/01"[PDAT] : "3000/12/31"[PDAT]))

Embase

http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=main&MODE=ovid&D=oemezd

((*"Fecal Microbiota Transplantation"/ OR "fecal microbiota transplantation".ti,ab OR "fecal microbiota transplantations".ti,ab OR "fecal microbiota transplant".ti,ab OR "fecal microbiota transplant*".ti,ab OR "fecal microbiota transfer".ti,ab OR "fecal microbiota transfer*".ti,ab OR "faecal microbiota transplantation".ti,ab OR "faecal microbiota transplant".ti,ab OR "faecal microbiota transplant*".ti,ab OR "faecal microbiota transfer".ti,ab OR "faecal microbiota transfer*".

ti,ab OR "Intestinal Microbiota Transfer".ti,ab OR "Intestinal Microbiota Transplantation".ti,ab OR "Intestinal Microbiota transplant".ti,ab OR "Intestinal Microbiota transplant*".ti,ab OR "Donor Feces Infusion".ti,ab OR "Donor Feces".ti,ab OR "Donor Faeces".ti,ab OR "Donor Fecal".ti,ab OR "Donor Faecal".ti,ab OR "fecal microbial transplantation".ti,ab OR "fecal microbial transplant".ti,ab OR "fecal microbial transplant*".ti,ab OR "fecal microbial transfer".ti,ab OR "fecal microbial transfer*". ti,ab OR "faecal microbial transplantation".ti,ab OR "faecal microbial transplant".ti,ab OR "faecal microbial transplant*".ti,ab OR "fecal transplantation".ti,ab OR "fecal transplant".ti,ab OR "fecal transplant*".ti,ab OR "fecal transfer".ti,ab OR "fecal transfer*".ti,ab OR "faecal transplantation".ti,ab OR "faecal transplant".ti,ab OR "faecal transplant*".ti,ab OR "fecal microbiome transplantation". ti,ab OR "fecal microbiome transplantations".ti,ab OR "fecal microbiome transplant".ti,ab OR "fecal microbiome transplant*".ti,ab OR "fecal microbiome transfer".ti,ab OR "fecal microbiome transfer*".ti,ab OR "faecal microbiome transplantation".ti,ab OR "faecal microbiome transplant*". ti,ab OR (("fecal microbiota".ti,ab OR "feces microbiota".ti,ab OR "faecal microbiota".ti,ab OR "faeces microbiota".ti,ab OR "fecal microb*".ti,ab OR "feces microb*".ti,ab OR "faecal microb*". ti,ab OR "faeces microb*".ti,ab) ADJ6 ("transplant*".ti,ab))) AND (exp *"microbial colonization"/ OR "colonization".ti,ab OR "colonisation".ti,ab OR "decolonization".ti,ab OR "decolonisation".ti,ab OR "coloniz*".ti,ab OR "colonis*".ti,ab OR "decoloniz*".ti,ab OR "decolonis*".ti,ab OR exp *"antibiotic resistance"/ OR "antibiotic resistance".ti,ab OR *"multidrug resistance"/ OR "multi-drug resistant". ti,ab OR "multi-drug resistance".ti,ab OR "multidrug resistant".ti,ab OR "multidrug resistance". ti,ab OR *"carbapenem"/ OR *"carbapenem derivative"/ OR "carbapenem".ti,ab OR "Carbapenem*". ti,ab OR *"Doripenem"/ OR *"Ertapenem"/ OR *"Thienamycins"/ OR *"Thienamycin"/ OR *"Imipenem"/ OR *"Meropenem"/ OR "Doripenem".ti,ab OR "Ertapenem".ti,ab OR "Thienamycins". ti,ab OR "Thienamycin".ti,ab OR "Imipenem".ti,ab OR "Meropenem".ti,ab OR "vancomycin".ti,ab OR *"Vancomycin"/ OR *"Vancomycin derivative"/ OR "Vancomycin*".ti,ab OR *"extended spectrum beta lactamase"/ OR "ESBL".ti,ab OR "extended spectrum".ti,ab OR "extendedspectrum".ti,ab OR "extended spectr*".ti,ab OR "extendedspectr*".ti,ab OR "multi-resistant".ti,ab OR "drug resistant". ti,ab OR "multi-resistance".ti,ab OR "drug resistance".ti,ab OR "multi-resistan*".ti,ab OR "drug resistan*".ti,ab) AND (2019 OR 2020 OR 2021 OR 2022).yr)

NOT conference review.pt
NOT (conference review or conference abstract).pt
AND (conference abstract).pt

Web of Science

http://isiknowledge.com/wos

((ti=("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation"

OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") AND ("transplant*"))) OR ab=("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") NEAR/6 ("transplant*"))) OR ak=("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") NEAR/6 ("transplant*")))) AND (ti=("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi-drug resistant" OR "multi-drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR

"Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*") OR ab=("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi-drug resistant" OR "multi-drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*") OR ak=("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi-drug resistant" OR "multi-drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*")) AND py=(2019 OR 2020 OR 2021 OR 2022))

Cochrane

https://www.cochranelibrary.com/advanced-search/search-manager

(("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*"

OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") AND ("transplant*"))) AND ("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi drug resistant" OR "multi drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*")):ti,ab,kw

AND py=(2019 OR 2020 OR 2021 OR 2022)

Emcare

http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=n&CSC=Y&PAGE=main&D=emcr

((*"Fecal Microbiota Transplantation"/ OR "fecal microbiota transplantation".ti,ab OR "fecal microbiota transplantations".ti,ab OR "fecal microbiota transplant".ti,ab OR "fecal microbiota transplant*".ti,ab OR "fecal microbiota transfer".ti,ab OR "fecal microbiota transfer*".ti,ab OR "faecal microbiota transplantation".ti,ab OR "faecal microbiota transplant".ti,ab OR "faecal microbiota transplant*".ti,ab OR "faecal microbiota transfer".ti,ab OR "faecal microbiota transfer*". ti,ab OR "Intestinal Microbiota Transfer".ti,ab OR "Intestinal Microbiota Transplantation".ti,ab OR "Intestinal Microbiota transplant".ti,ab OR "Intestinal Microbiota transplant*".ti,ab OR "Donor Feces Infusion".ti,ab OR "Donor Feces".ti,ab OR "Donor Faeces".ti,ab OR "Donor Fecal".ti,ab OR "Donor Faecal".ti,ab OR "fecal microbial transplantation".ti,ab OR "fecal microbial transplant".ti,ab OR "fecal microbial transplant*".ti,ab OR "fecal microbial transfer".ti,ab OR "fecal microbial transfer*". ti,ab OR "faecal microbial transplantation".ti,ab OR "faecal microbial transplant".ti,ab OR "faecal microbial transplant*".ti,ab OR "fecal transplantation".ti,ab OR "fecal transplant".ti,ab OR "fecal transplant*".ti,ab OR "fecal transfer".ti,ab OR "fecal transfer*".ti,ab OR "faecal transplantation".ti,ab OR "faecal transplant".ti,ab OR "faecal transplant*".ti,ab OR "fecal microbiome transplantation". ti,ab OR "fecal microbiome transplantations".ti,ab OR "fecal microbiome transplant".ti,ab OR "fecal microbiome transplant*".ti,ab OR "fecal microbiome transfer".ti,ab OR "fecal microbiome transfer*".ti,ab OR "faecal microbiome transplantation".ti,ab OR "faecal microbiome transplant*". ti,ab OR (("fecal microbiota".ti,ab OR "feces microbiota".ti,ab OR "faecal microbiota".ti,ab OR "faeces microbiota".ti,ab OR "fecal microb*".ti,ab OR "feces microb*".ti,ab OR "faecal microb*". ti,ab OR "faeces microb*".ti,ab) ADJ6 ("transplant*".ti,ab))) AND (exp *"microbial colonization"/ OR "colonization".ti,ab OR "colonisation".ti,ab OR "decolonization".ti,ab OR "decolonisation".ti,ab OR "coloniz*".ti,ab OR "colonis*".ti,ab OR "decoloniz*".ti,ab OR "decolonis*".ti,ab OR exp *"antibiotic resistance"/ OR "antibiotic resistance".ti,ab OR *"multidrug resistance"/ OR "multi-drug resistant". ti,ab OR "multi-drug resistance".ti,ab OR "multidrug resistant".ti,ab OR "multidrug resistance". ti,ab OR *"carbapenem"/ OR *"carbapenem derivative"/ OR "carbapenem".ti,ab OR "Carbapenem*". ti,ab OR *"Doripenem"/ OR *"Ertapenem"/ OR *"Thienamycins"/ OR *"Thienamycin"/ OR

*"Imipenem"/ OR *"Meropenem"/ OR "Doripenem".ti,ab OR "Ertapenem".ti,ab OR "Thienamycins".ti,ab OR "Thienamycin".ti,ab OR "Imipenem".ti,ab OR "Meropenem".ti,ab OR "vancomycin".ti,ab OR *"Vancomycin"/ OR *"Vancomycin derivative"/ OR "Vancomycin*".ti,ab OR *"extended spectrum beta lactamase"/ OR "ESBL".ti,ab OR "extended spectrum".ti,ab OR "extendedspectrum".ti,ab OR "extended spectr*".ti,ab OR "extendedspectr*".ti,ab OR "multi-resistant".ti,ab OR "drug resistant".ti,ab OR "multi-resistance".ti,ab OR "drug resistance".ti,ab OR "multi-resistan*".ti,ab OR "drug resistan*".ti,ab) AND (2019 OR 2020 OR 2021 OR 2022).yr)

Academic Search Premier

http://search.ebscohost.com/login.aspx?authtype=ip,uid&profile=lumc&defaultdb=aph

TI(("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") AND ("transplant*"))) AND ("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi drug resistant" OR "multi drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*")) OR SU(("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation"

OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*") AND ("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi drug resistant" OR "multi drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*")) OR KW(("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR "fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*") AND ("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi drug resistant" OR "multi drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*")) OR (TI("Fecal Microbiota Transplantation" OR "fecal microbiota transplantation" OR "fecal microbiota transplantations" OR "fecal microbiota transplant" OR "fecal microbiota transplant*" OR "fecal microbiota transfer" OR

"fecal microbiota transfer*" OR "faecal microbiota transplantation" OR "faecal microbiota transplant" OR "faecal microbiota transplant*" OR "faecal microbiota transfer" OR "faecal microbiota transfer*" OR "Intestinal Microbiota Transfer" OR "Intestinal Microbiota Transplantation" OR "Intestinal Microbiota transplant" OR "Intestinal Microbiota transplant*" OR "Donor Feces Infusion" OR "Donor Feces" OR "Donor Faeces" OR "Donor Fecal" OR "Donor Faecal" OR "fecal microbial transplantation" OR "fecal microbial transplant" OR "fecal microbial transplant*" OR "fecal microbial transfer" OR "fecal microbial transfer*" OR "faecal microbial transplantation" OR "faecal microbial transplant" OR "faecal microbial transplant*" OR "fecal transplantation" OR "fecal transplant" OR "fecal transplant*" OR "fecal transfer" OR "fecal transfer*" OR "faecal transplantation" OR "faecal transplant" OR "faecal transplant*" OR "fecal microbiome transplantation" OR "fecal microbiome transplantations" OR "fecal microbiome transplant" OR "fecal microbiome transplant*" OR "fecal microbiome transfer" OR "fecal microbiome transfer*" OR "faecal microbiome transplantation" OR "faecal microbiome transplant*" OR (("fecal microbiota" OR "feces microbiota" OR "faecal microbiota" OR "faeces microbiota" OR "fecal microb*" OR "feces microb*" OR "faecal microb*" OR "faeces microb*") AND ("transplant*"))) AND AB("microbial colonization" OR "colonization" OR "colonisation" OR "decolonization" OR "decolonisation" OR "coloniz*" OR "colonis*" OR "decoloniz*" OR "decolonis*" OR "antibiotic resistance" OR "antibiotic resistance" OR "multidrug resistance" OR "multi drug resistant" OR "multi drug resistance" OR "multidrug resistant" OR "multidrug resistance" OR "carbapenem" OR "carbapenem derivative" OR "carbapenem" OR "Carbapenem*" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "Doripenem" OR "Ertapenem" OR "Thienamycins" OR "Thienamycin" OR "Imipenem" OR "Meropenem" OR "vancomycin" OR "Vancomycin" OR "Vancomycin derivative" OR "Vancomycin*" OR "extended spectrum beta lactamase" OR "ESBL" OR "extended spectrum" OR "extendedspectrum" OR "extended spectr*" OR "extendedspectr*" OR "multi-resistant" OR "drug resistant" OR "multi-resistance" OR "drug resistance" OR "multi-resistan*" OR "drug resistan*"))

Supplementary Table 1: Overview of ongoing trials.

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT04431934	Randomised, open-label, controlled trial	437	November 2020	December 2022	Adults with documented rectal colonisation with multidrug resistant gram negative bacteria, eligible for routine digestive decolonisation	7 days of non-absorbable antibiotics followed by: **Group 1**: FMT 2 doses, once a week, 14-17 capsules per dose (dose is equivalent to 50 gr of healthy donor stool) **Group 2**: 2 sachets of probiotics every 12 hours for 14 days **Group 3**: no intervention	Decolonisation rate, defined as negative rectal swab, after 60 days
NCT04188743	Randomised, double-blind, controlled trial	150	December 2019	December 2023	Adults with at least two consecutive confirmations of MDRO colonisation in faeces	**Group 1**: allogenic FMT: 50 gr of healthy donor stool, frozen, administered by nasoduodenal tube **Group 2**: autologous FMT: 50 gr of own stool, frozen, administered by nasoduodenal tube **Group 3**: no intervention	Decolonisation rate, defined as three consecutive negative stool cultures in minimal time span of 2 weeks, after 1 month after treatment

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT04146337	Randomised, open-label, controlled trial	60	October 2020	June 2022	Adult inpatients positive for CRE of any strain and resistance mechanism in rectal surveillance stool samples, with or without CRE clinical samples. A positive rectal swab within one week before randomisation is mandatory.	**Group 1**: FMT, 15 capsules a day for two consecutive days after an eight hour fast **Group 2**: no intervention	Decolonisation rate, defined as three consecutive negative rectal cultures, at 28 days
NCT04760665	Randomised, double-blind, controlled trial	120	April 2021	July 2022	Adult patients colonised with KPC-producing *Klebsiella pneumoniae* (undefined), without an active infection in the month prior to inclusion	**Group 1**: four oral capsules containing healthy donor faeces **Group 2**: four oral placebo capsules	Decolonisation rate (undefined) at 30 days
EUCTR2019-004402-10-FR	Randomised, double-blind, controlled trial	214	Not mentioned	Not mentioned	Adult patients colonised with ESBL-E or CRE, assessed with stool culture, and having suffered from an infection with ESBL-E in the previous 12 months	**Group 1**: FMT capsules (n= 25) for two days in a row **Group 2**: placebo	Decolonisation rate at 30 days, determined by (undefined) culture methods

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT04746222	Randomised, double-blind, controlled trial	108	July 2021	July 2023	Adults (age ≥ 21) colonisation with CRE, confirmed with at least one positive rectal swab (PCR) taken ≤7 days before randomisation. Antibiotics ceased for at least 48 hours pre-randomisation evaluation.	**Group 1**: single dose of 30 oral capsules containing healthy donor stool **Group 2**: single dose of 30 placebo capsules	Decolonisation rate, defined by negative rectal swab (PCR/ culture), at 12 weeks
NCT04759001	Randomised, double-blind, controlled trial	52	February 2021	February 2023	Adults with CRE colonisation, confirmed by a rectal swab	**Group 1**: FMT by colonoscopy with healthy donor stool **Group 2**: placebo (water) administered through colonoscopy	Decolonisation rate, defined by negative rectal swab, at 4 weeks
NCT04181112	Randomised, open-label, controlled trial	90	November 2019	November 2023	Adult renal transplant recipients, colonised with a multidrug resistant organism (undefined), confirmed by rectal swab or stool culture	**Group 1**: FMT using retention enema **Group 2**: Antibiotic pretreatment (undefined) followed by FMT using retention enema **Group 3**: no intervention	Decolonisation rate, defined by negative culture/PCR at 14 and 30 days post-FMT

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT03802461	Randomised, open-label, controlled trial	40	March 2019	December 2020 (no published data yet)	Adults with ≥ 1 rectal swab, groin, stool, or urine specimen positive for CRE within the past month	**Group 1**: bowel lavage followed by FMT (50 gr healthy donor stool) administered by enema, given on 3 occasions **Group 2**: no intervention	Decolonisation rate (undefined) after 3 months
EUCTR2019-001618-41	Randomised, participant-blinded, controlled, feasibility trial	80	September 2019	March 2022	Adults with documented gastrointestinal carriage of ESBL-E or CRE (stool sample) in the 21 days prior to consent **and** symptomatic infection with the target organism in the preceding 6 months	**Group 1**: FMT capsules (80 gr of healthy donor faeces per 5 capsules) on three consecutive days. Pretreatment with proton-pump inhibitor **Group 2**: Placebo capsules	To determine the feasibility and acceptability of administering encapsulated FMT to participants colonised with ESBL-E/CPE. This will be used to determine if a substantive trial is feasible. A secondary objective is to provide early evidence of **efficacy** (decolonisation rate by culture/PCR at days 10, 40, 100, and 190)

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT03063437	Randomised, double-blind, controlled trial	9 currently enrolled	August 2017	February 2019 **Preliminary results:** VRE decolonisation at day 10: 1 out of 4 participants in FMT group, and 1 out of 5 participants in placebo group	Adults colonised with VRE (by stool culture) in the last 14 days	**Group 1**: Single dose of FMT (30 capsules per dose) **Group 2**: Placebo capsules	VRE decolonisation rate (absence of VRE on stool culture) at day 10
NCT02922816	Randomised, open-label, controlled trial	20	December 2016	June 2021 (no published data yet)	Adult renal transplant recipients with a history of MDRO infection	**Group 1**: FMT via enema, healthy donor faeces, 2 cycles of 6 weeks, pretreatment with magnesium citrate **Group 2**: pretreatment like group 1, but no FMT. Participants can cross-over to FMT group after one cycle	Decolonisation rate (rectal swab or stool culture) at day 36

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT03061097	Randomised, double-blind, controlled trial	4 (of 20 estimated) participants currently enrolled	July 2017	June 2019 **Preliminary results:** 0 out of 4 patients were decolonised 28 days after autologous FMT	Long-term care residents with a history of an infection requiring antimicrobial treatment at the discretion of the treating physician	**Group 1**: Autologous 125 mL FMT (biobanked stool from same patient collected before infection requiring antibiotics) via enema **Group 2**: Placebo FMT	Safety (short-term) at Day 7 defined as NIH Grade ≥2 adverse events. Secondary objective: among patients with MDRO colonisation at day 0: decolonisation rate at day 3, day 7 and day 28
NCT02312986	Prospective cohort study, single-group	20	August 2015	July 2020 **Preliminary results:** Data available for 1 participant: had an MDRO infection at 6 months post FMT	Adults with a history of at least three recurrent infections due to an MDRO; at least two recurrent, severe infections due to MDRO requiring hospitalisation; or at least two recurrent infections due to MDRO for which only antimicrobials with rate limiting toxicities are available AND the MDRO is likely of enteric origin.	FMT (150 mL) via enema, no further information	Incidence of adverse events within 12 months of FMT. Secondary outcome: number of subjects with MDRO infections 30 days, 6 months and 12 months post-FMT

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT02543866	Prospective cohort study, single group	20	February 2017	September 2024	Children and adolescents with a history at least one infection due to pathogens non-susceptible to ceftriaxone, cefotaxime, or ceftazidime	FMT (50 mL) via nasogastric tube, no further information	Incidence of adverse events within 12 months of FMT. Secondary outcome: number of subjects free from MDRO intestinal colonisation and recurrent MDRO infections 2 days, 2 weeks, 4 weeks, 8 weeks, 6 months, and 12 months post-FMT
NCT03167398	Prospective cohort study, single-group	15	February 2018	December 2019 (no published data yet)	Adult inpatients for CRE of any strain and resistance mechanism in rectal surveillance stool samples, with or without CRE clinical samples. A positive rectal swab within one week before randomisation will be mandatory	Capsulised FMT: 15 capsules a day for two consecutive days. Pretreatment with proton pump inhibitor (and during FMT treatment)	Decolonisation rate, defined by three consecutive negative rectal samples, after 1 month

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT03367910	Prospective cohort study, single-group	60	February 2018	December 2021	Adults with a history of at least three recurrent infections due to an MDRO; at least two recurrent, severe infections due to MDRO requiring hospitalisation; or at least two recurrent infections due to MDRO for which only antimicrobials with rate limiting toxicities are available	FMT (150 mL) via enema, no further information	Incidence of adverse events within 6 months of FMT. Secondary outcome: risk of recurrent UTI 6 months post-FMT and MDRO decolonisation (stool and urine specimens) 6 months post-FMT
NCT03029078	Prospective cohort study, single-group	50	November 2014	January 2024	Adults patients colonised with VRE or CRE, confirmed by at least three positive swabs in the last month	FMT via nasoduodenal tube with healthy donor faeces. Pretreatment with bowel lavage	Decolonisation rate (undefined) at 1 week, 2 weeks, 1 month and 6 months
NCT03479710	Prospective cohort study, with control group, non-randomised, open-label	40	February 2018	December 2021	Adult patients colonised with VRE or CRE, confirmed by two or more stool or rectal swabs at least one week apart	**Group 1**: FMT (100-200 mL) via nasoduodenal tube, with frozen donor stool **Group 2**: no intervention	Decolonisation rate (undefined) at 2 weeks and 12 months
NCT04583098	Prospective cohort study, single-group	100	March 2019	March 2022	Adults colonised with VRE or CRE (undefined)	FMT (route of administration not mentioned) with frozen stool from healthy donors	Decolonisation rate, confirmed by 3 negative rectal swab cultures with a 3 day interval, at 3 months post-FMT

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT04593368	Prospective cohort study, single-group	15	December 2020	September 2023	Children and adults (aged 3-25) with an indication for allogeneic haematopoietic stem cell transplantation and colonisation with VRE, ESBL-E, *Acinetobacter* spp., MRSA, *Stenotrophomonas* spp., *S. viridans*, *C. difficile* or *Pseudomonas aeruginosa*	FMT (oral, exact route of administration not mentioned) from allogeneic donor, 0.5-2 g/kg of recipients weight	Decolonisation rate (undefined) 7 days after FMT
NCT04790565	Prospective cohort study, single-group	60	April 2021	April 2023	Adults with CRE colonisation in surveillance stool samples, with or without clinical CRE samples	FMT, 15 capsules a day for two consecutive days after an eight hour fast	Decolonisation rate, defined as 3 consecutive negative rectal cultures, at 28 days
NCT03834051	Prospective cohort study, single-group	50	February 2019	August 2020 (no published data yet)	Adults with ESBL-E, CRE or VRE colonisation (undefined)	FMT via enema, no further information	Decolonisation rate, time frame: 2 years

Supplementary Table 1: Continued

NCT/EUCTR number	Study design	Estimated enrolment (n)	Start date	Estimated completion (and preliminary results if posted)	Inclusion criteria	Arms and Interventions	Primary outcome (secondary outcome is mentioned if relevant)
NCT03050515	Prospective cohort study, single-group	12	February 2018	February 2020 (no published data yet)	Female patients (aged > 18) with recurrent urinary tract infections (2 or more culture proven in last 6 months) failing with oral prophylaxis or intravesical instillations with dimethylsulfoxide or heparin/lidocaine	FMT via enema with donor stool, pretreatment with bowel lavage	Change in frequency of culture proven urinary tract infections at 6 months post-FMT

Abbreviations: FMT = faecal microbiota transplantation, MDRO = multidrug resistant organism, CRE = carbapenemase resistant Enterobacteriaceae, KPC = *Klebsiella pneumoniae* carbapenemase, ESBL-E = extended spectrum beta-lactamase producing Enterobacteriaceae, PCR = polymerase chain reaction, VRE = vancomycin resistant *Enterococcus*, MRSA = methicillin resistant *Staphylococcus aureus*

Supplementary Table 2: Risk of bias assessment.

First author & year	Representativeness of exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough for outcome to occur	Adequacy of follow-up
Lee 2020 Korea (25)	**High risk of bias** Characteristics of exposed cohort not described	**High risk of bias** Characteristics of non-exposed cohort not described	**High risk of bias** Not adequately described	**Low risk of bias**	**High risk of bias** Comparability of cohorts could not be assessed because cohorts were not described	**High risk of bias** Assessment of outcome not reported	**Low risk of bias**	**High risk of bias** Many patients lost to follow-up and no description of those lost
Bar-Yoseph 2020 Israel (26)	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**High risk of bias** Significantly more patients in control group had systemic antibiotics and prolonged hospital stay post-FMT.	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**
Ghani 2020 United Kingdom (23)	**High risk of bias** Mostly immunocompromised patients and significant antibiotic use pre- and post-FMT	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**High risk of bias** Not reported for three patients in group 1 and 2, not reported for control group at all	**Low risk of bias**	**High risk of bias** Some dropouts, but no description provided

Supplementary Table 2: Continued

First author & year	Representativeness of exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough for outcome to occur	Adequacy of follow-up
Seong 2020 Korea (24)	**High risk of bias** Significant antibiotic use pre- and post-FMT	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**
Lee 2020 Korea (28)	**High risk of bias** Inclusion of some rCDI patients	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**
Merli 2020 Italy (29)	**High risk of bias** Exclusively immunocompromised children	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**
Silva 2020 Portugal (27)	**High risk of bias** Concurrent rCDI	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Not applicable**	**Low risk of bias**	**Low risk of bias**	**Low risk of bias**

Abbreviations: FMT = faecal microbiota transplant, rCDI = recurrent *Clostridioides difficile* infection

Chapter 8

General discussion

The general aim of this thesis was to investigate current issues with the definition, diagnosis and treatment of urinary tract infection (UTI). The rationale for wanting to improve these aspects of UTI primarily lies in the significant physical and emotional burden faced by patients suffering from UTI. Beyond the burden on the individual patient, the high incidence of UTI puts a considerable strain on all layers of the health care system. The urgency to address these issues has only increased, given the escalating threat of antimicrobial resistance (AMR) to public health. The emergence of multidrug resistant organisms (MDROs) outpaces the development of novel antimicrobials. UTI is a key driver of AMR, not only due to its high incidence and tendency to recur, but also due to the inaccuracy of current urine diagnostics, particularly in older women. One of the root causes of inappropriate antimicrobial treatment is inappropriate diagnosis. Therefore, efforts to combat AMR should not only focus on developing novel antimicrobials, but also on improving diagnostics to support judicious antimicrobial use. Given the challenges in symptom assessment and the high prevalence of asymptomatic bacteriuria (ASB) in older women, accurate diagnostics for UTI are arguably most needed in this population. MDRO carriership is common in older adults, facilitating the spread of MDROs in the community, hospitals, and long-term care facilities (LTCF). [1, 2] To generate new, reliable data on novel diagnostics and treatment modalities for UTI, clear research definitions of UTI (and its various clinical phenotypes) are of paramount importance. Without an agreed reference standard, the internal and external validity of such studies is compromised.

This general discussion addresses the challenges related to the definition, diagnosis and treatment of UTI in three parts. For each part, the results of the studies in this thesis will be discussed, including implications for future research.

Part I: Defining UTI

Chapter 2 describes the results of a systematic review evaluating how UTI has been defined in recent studies. In total, 47 studies, published between 2019 and 2022, investigating prophylactic and therapeutic interventions in adults with UTI, were included. UTI definitions used in these studies were highly heterogeneous, consisting of various combinations of clinical signs and diagnostic tests (or a lack thereof). There are several factors that may explain this heterogeneity.

Firstly, the diverse clinical presentations and manifestations of UTI, taken together with a certain degree of subjectivity in symptoms, may lead to variations

in how researchers define UTI. As already mentioned in the introduction of this thesis, UTI is not a single clinical entity, but rather refers to a spectrum of disease manifestations. Secondly, there is a lack of consensus within the scientific community regarding thresholds for 'significant' pyuria and bacteriuria. This disagreement among experts was reflected in the various thresholds for pyuria and bacteriuria we found in the studies included in our systematic review. Thirdly, studies with distinct objectives (e.g. clinical trials and diagnostic accuracy studies) may use different criteria tailored to their specific research goals. A clinical trial evaluating the efficacy of a novel antimicrobial may define UTI based on the intended use of the antimicrobial for a specific population, e.g. including fever in the definition of UTI if the study drug has systemic properties. Diagnostic accuracy studies may define UTI based on more precise laboratory criteria. As our systematic review only included interventional studies, we could not deduce this from our data.

Differences between existing research guidelines may have led to conflicting definitions. European Medicines Agency (EMA) [3] and U.S. Food and Drug Administration (FDA) [4, 5] guidelines apply different symptom and laboratory criteria, leaving room for interpretation. Definitions of 'complicated UTI' are not uniform in these guidelines. In our systematic review, we found that 'complicated UTI' referred to two different clinical entities, i.e. UTI with systemic involvement and UTI with complicating host factors, likely due to the ambiguity of this term. However, the diversity observed in study definitions within our systematic review cannot be solely ascribed to conflicting guidelines, as the overall adherence to these guidelines proved to be low.

This leads us to question why adherence to existing guidelines is generally low. Apart from the lack of clarity within and cohesiveness between these guidelines, these guidelines were developed to facilitate clinical development programmes for novel antimicrobials or new uses and/or regimens for licensed antimicrobials. As such, researchers conducting studies for other purposes, e.g. evaluating novel diagnostic tests, may not feel compelled to follow these guidelines for their specific study objectives. Moreover, if there are no institutional or journal requirements mandating the use of specific guidelines or definitions, researchers may choose more flexible or alternative approaches. Other existing research guidelines are limited in their applicability, as they were developed for surveillance purposes or for studies conducted in specific settings, such as LTCFs. [6, 7]

The findings of our systematic review led us to establish a reference standard for UTI, intended for research purposes rather than clinical practice. As previously noted in the introduction section of this thesis, a reference standard is crucial for identification of homogeneous groups of patients for clinical research. Without a reference standard, bias is introduced into estimates of diagnostic accuracy and efficacy, affecting the internal validity of a study, and results cannot be readily compared with other studies (or synthesised for meta-analysis), compromising its external validity. Moreover, a reference standard creates a common language for international researchers.

We conducted a Delphi study, described in **Chapter 3** of this thesis, to achieve consensus on a reference standard. Used in various fields, the Delphi method has four main characteristics: an expert panel is questioned about the issue of interest, the process is anonymous to reduce the effect of dominant personalities, the questionnaires are iterative in nature, and the design of the subsequent rounds is informed by a summary of the group response of the previous round. [8] This study included 57 UTI experts from various countries across Europe and North America, representing medical specialties including infectious diseases, urology, microbiology, geriatrics, family medicine, and emergency medicine. After three questionnaire rounds, a high degree of consensus (94%) on the final reference standard was reached.

There are some notable differences between this reference standard and the aforementioned research guidelines. UTI diagnosis involves many factors, and in clinical practice there are levels of probability when diagnosing UTI. To reflect this, our reference standard includes a scoring system with possible, probable, and definite UTI categories, echoing the categorisation that can be found in the now widely-used *European Organisation for Research and Treatment of Cancer and the Mycoses Study Group* (EORTC/MSG) consensus definitions of invasive fungal diseases. [9] For clarity purposes, our reference standard steers away from the term 'complicated UTI' and instead distinguishes between UTI with and without systemic involvement. We chose this distinction to align more closely with clinical practice and to ensure that future UTI studies should then be able to focus upon clearly phenotyped cohorts. For instance, a recent randomised trial comparing a novel aminoglycoside to meropenem for the treatment of 'complicated UTI', applied the FDA definition, in which 'complicated' may either refer to UTI with systemic signs (e.g. fever) or UTI with complicating host factors (e.g. urological comorbidity). As a consequence, they included a heterogeneous group of study

participants with and without systemic involvement and various complicating host factors. [10] Aside from the probably unnecessary treatment of acute cystitis with broad-spectrum intravenous antibiotics in some study participants, this limits the interpretation of study results and the external validity of the study.

Another major difference between our reference standard and previous guidelines is the incorporation of different levels of pyuria. This decision was primarily based on study outcomes described in **Chapter 4**, in which we demonstrate that the low pyuria thresholds used in previous guidelines have low specificity for UTI in older women, and the degree of pyuria can help to distinguish UTI from ASB in this population. Considering the predominance of UTI in older women and the high need for reliable data in this understudied population, we believed that a new reference standard should take the high prevalence of ASB in older women into account and integrated this into our scoring system for pyuria (and bacteriuria) domains.

Despite compelling evidence that the absence of pyuria effectively rules out UTI [11, 12], our systematic review (**Chapter 2**) showed that pyuria was seldomly incorporated into study definitions, and if it was, the presence of leukocyte esterase on a urine dipstick was usually considered sufficient. As leukocyte esterase results exhibit poor correlation with absolute degrees of pyuria, and the quantification of pyuria is crucial for enhancing comparability across future studies, our reference standard is based on leukocyte quantification and omits urine dipstick items. While quantification of pyuria should also be encouraged in UTI studies conducted in primary and long-term care settings, our supplementary reference standard does include urine dipstick items, to ensure the broad applicability of our reference standard. Finally, our reference standard applies lower bacteriuria thresholds than any of the aforementioned standards. This decision was based on clear expert panel consensus and previous evidence demonstrating lower colony-counts in 'clear-cut' cases of UTI. [11, 12] The multifaceted scoring system of our reference standard mitigates the risk of a lower bacteriuria threshold leading to misclassification of ASB as UTI.

The open-ended question is whether our reference standard will be implemented in future UTI studies. While the low adherence to previous standards suggests a need for a new reference standard, the implementation of our reference standard is not assured. However, several aspects of our reference standard increase the likelihood of successful implementation. Firstly, by involving a large and diverse

group of stakeholders, we incorporated viewpoints from multiple different medical specialties and countries, increasing applicability and endorsement of the reference standard. The same approach has resulted in the widespread adoption of consensus definitions for invasive fungal diseases in major trials assessing antifungal drug efficacy, validation studies of diagnostic tests, and epidemiological research. [13] Similarly, our reference standard is versatile and applicable across various study types, in contrast to the EMA and FDA standards, which were specifically developed for the approval of new antimicrobials. Another strength of our reference standard lies in its clarity, specifically, its avoidance of ambiguous terms such as 'complicated UTI'. Ideally, the use of our reference standard would serve as a quality criterion for journals and ethical committees. It is important to reiterate that our reference standard was not developed for clinical practice and should, therefore, not be utilised in such settings. Our reference standard was not validated for clinical use and does not consider the practical aspects of clinical practice. For instance, a urine culture result may not be available at the time of patient presentation.

To ensure continued use of the reference standard in future studies, the reference standard will have to be updated once new evidence emerges. For instance, if the novel urine biomarkers described in **Chapter 5** of this thesis will have been validated in a broader population, they could be integrated in an updated reference standard. Additionally, further calibration of the reference standard in future studies may result in adjustments to certain domains or the weighting of specific criteria. While our reference standard was partially validated and calibrated using fictional case vignettes, future studies could involve a more extensive and diverse set of case vignettes. Alternatively, they could assess the alignment of the reference standard with actual clinical cases, as adjudicated by a separate expert panel.

Part II: Diagnostic challenges

As summarised in the introduction of this thesis, diagnosing UTI is perhaps most challenging in older women. One approach to addressing these diagnostic challenges in clinical practice is by examining how existing diagnostic tests can be optimised.

Due to their convenience, urine dipsticks are frequently used in primary care and LTCF settings, but they lack accuracy. [14] While automated microscopy and

urine flow cytometry are more precise methods for quantification of pyuria, currently applied reference values do not take the high prevalence of ASB (with concomitant pyuria) in older women into account. In **Chapter 4**, we describe the results of a case-control study, conducted across multiple primary care offices, LTCFs and emergency departments, in which we evaluated the diagnostic accuracy of automated microscopy and urine flow cytometry for UTI in women ≥ 65 years. Our main findings were as follows: both diagnostic methods had (equally) high diagnostic accuracy for UTI in this population, the level of pyuria could aid in distinguishing UTI from ASB, and the specificity of the commonly used pyuria threshold (10 leukocytes/µl) for UTI was poor (36%). These results are difficult to compare with prior studies, as they generally use the presence of bacteriuria as a proxy reference standard for UTI, which does not distinguish UTI from ASB. As has been stated, ramifications of inappropriately diagnosing UTI include antimicrobial overtreatment and failing to address the true cause of symptoms. In our study, a threshold of 200 leukocytes/µl increased the specificity to 86% (95% confidence interval (CI) 78 – 92), while maintaining a high sensitivity of 89% (95%CI 80 – 96), corresponding with a positive likelihood ratio of 6.3 (95%CI 3.9 – 10.3) and a negative likelihood ratio of 0.1 (95%CI 0.06 – 0.3).

The potential consequences of these findings for clinical practice differ per health care setting. In hospitals, pyuria is usually quantified (via automated microscopy or urine flowcytometry) in patients with suspected UTI, although some laboratories forego quantification if initial dipstick screening does not yield abnormal results. While the latter diagnostic strategy could contribute to underdiagnosis of UTI, the primary concern in older women is overdiagnosis, or rather, inappropriate diagnosis. Based on our findings in **Chapter 4** and the widespread availability of pyuria quantification in most hospitals, we propose that pyuria should be quantified in all women ≥ 65 years with suspected UTI in this setting, and a higher threshold (e.g. 200 leukocytes/µl) should be employed. Alternatively, the test result could be accompanied by a message reminding the ordering clinician that intermediate degrees of pyuria are also found in older women with ASB. Evidently, a one-size-fits-all threshold for pyuria is a concept better left in the past, and age-, sex-, and setting-specific reference values warrant further study.

However, the majority of women with suspected UTI present in primary care, and rates of ASB are highest in women residing in LTCF. [15, 16] Therefore, the potential impact of pyuria quantification and novel thresholds is arguably highest in these healthcare settings. Nevertheless, there are feasibility concerns

to address. While automated microscopy has the advantage of a reduced labour intensity, reduced interobserver variability, and higher throughput than manual microscopy, automated microscopy still requires some preanalytical steps and trained personnel to operate and maintain these automated systems. The Dutch primary care guideline on UTI [17] does recommend manual microscopy in case of leukocyte-esterase-positive and nitrite-negative urine dipstick results, but favours a urine dipslide (i.e. a slide coated with agar media for bacteriuria determination) in this scenario due to the ease-of-use. Alternatively, primary care physicians and geriatricians could send urine samples to central laboratories for pyuria quantification. However, this approach incurs additional financial and logistical costs, as pyuria quantification should be performed within a few hours for reliable results. [18]

In light of the feasibility challenges related to pyuria quantification in primary care offices and LTCFs, novel biomarkers are particularly needed in these settings. In **Chapter 5**, we have explored the diagnostic potential of twelve urine biomarkers in the same study population as described in **Chapter 4**. Urine biomarker concentrations were measured through liquid chromatography-mass spectrometry (LC-MS) and enzyme-linked immunosorbent assay (ELISA). We identified five urine biomarkers with high diagnostic accuracy for UTI in older women. Urinary interleukin 6 (IL-6), azurocidin, neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinases 2 (TIMP-2), and C-X-C motif chemokine 9 (CXCL-9) accurately differentiated older women with UTI from asymptomatic women, including those with ASB. Azurocidin exhibited the highest diagnostic accuracy (sensitivity 86% and specificity 89% at a cut-off of 16.7 ng/mmol creatinine). These biomarkers all play different roles in the innate immune response. [19-21] Interestingly, patients with ASB exhibited higher biomarker concentrations than asymptomatic patients without bacteriuria, but lower concentrations than patients with UTI, suggesting that ASB may cause a state of low-grade inflammation. A similar distribution was seen for pyuria concentrations in **Chapter 4**. IL-6 and azurocidin have been studied most extensively in this population, and our findings are consistent with prior studies. [22, 23]

However, some hurdles must be overcome before these novel biomarkers can be used in routine clinical practice, especially in non-hospital settings. Currently, these biomarkers are measured using ELISA and LC-MS. These methods are costly and require trained laboratory technicians. Less expensive and easier tests will

need to be developed, for instance in the form of point-of-care testing. A point-of-care test is a test that can be rapidly and easily performed at the patient's bedside. [24] These tests are already being developed. For instance, the Utriplex test is a point-of-care urine dipstick test measuring human neutrophil elastase, matrix metalloproteinase 8 and cystatin C. Its diagnostic accuracy was disappointing in a prior paediatric study, likely explained by misclassification as a result of their reference standard ('acute illness' with urine culture yielding a uropathogen ≥ 105 CFU/mL). However, this study illustrates that point-of-care testing for novel urinary inflammatory markers is feasible. [25]

Alternatively, rather than replacing pyuria as the keystone of UTI diagnosis, these new biomarkers could also be utilised in conjunction with pyuria to improve diagnostic accuracy. For instance, in a post hoc analysis in **Chapter 5** we found that when comparing UTI and ASB subgroups, the combination of several urine biomarkers with pyuria had superior diagnostic accuracy to pyuria alone. This is due to the fact that patients with ASB showed a rather wide range of pyuria.

Regardless of which diagnostic strategy will prove to be best, both in terms of accuracy and feasibility, our findings on both pyuria quantification and novel biomarkers need to be externally validated in a broader population with various clinical presentations (including non-specific symptoms), comorbidities and levels of frailty. Our case-control design and our study population were chosen to prove a concept for which a clear definition and reliable assessment of UTI and ASB was necessary but may have contributed to overestimated diagnostic accuracy results. Furthermore, our population was rather young and did not have advanced cognitive impairment, raising the question of whether our results apply to a more frail population. Future studies should also evaluate the impact of improved diagnostic accuracy on prescribing rates, MDRO colonisation rates, and patient outcomes (i.e. symptom burden).

Something we did not address in this thesis, but that may become increasingly important in the near future is reducing the turnaround time of pathogen identification and antimicrobial susceptibility testing (AST). Increasing AMR rates underscore the necessity of rapid AST results, allowing for tailored antimicrobial therapy. This is especially relevant in populations with high a priori probabilities of AMR, such as patients in LTCFs and patients with recurrent UTI. However, fast pathogen identification and susceptibility results can potentially lower the threshold for clinicians to prescribe (reserve) antimicrobials. While fast culture

results offer advantages in timely antimicrobial treatment, the associated risks underscore the importance of diagnostic tools capable of effectively differentiating between ASB and UTI.

Part III: Alternative prophylactic and treatment strategies

We have seen how ambiguous definitions and imprecise diagnostics of UTI contribute to inappropriate prescribing of antimicrobials, and thus to unnecessary side effects, drug interactions, *Clostridioides difficile* infections, and AMR. Patients with recurrent UTI (rUTI), defined as at least three episodes per year or two episodes per six months, particularly contribute to AMR, both due to frequent courses of antimicrobials and the use of continuous oral antimicrobial prophylaxis. [26] At the same time, patients with rUTI are disproportionally affected by the negative effects of AMR, as it limits their treatment options, sometimes precluding any oral antimicrobials. In healthy individuals, the gut microbiota, consisting of diverse communities of bacteria and other microorganisms, prevents the overgrowth of potentially harmful pathogens, also known as colonisation resistance. Antimicrobial treatment leads to a perturbed gut microbiota with impaired colonisation resistance, increasing the risk of invasive infections. These infections then require antimicrobial treatment, promoting further gut dysbiosis and selection of resistant strains. [27, 28] Worby et al. [28] have shown decreased gut microbial richness in women with rUTI. **Chapter 6 and 7** of this thesis focus on breaking this vicious cycle, which is displayed in **Figure 1**.

The direct instillation of antimicrobials in the bladder may be an appealing 'gut-sparing' alternative to systemic antimicrobial prophylaxis and treatment. For instance, in bacterial conjunctivitis, antimicrobial eye-drop formulations ensure a directly delivery of the drug to the site where it is needed, minimising systemic effects. Adjuvant chemotherapy in non-muscle invasive bladder cancer showcases the usefulness of targeted bladder delivery. [29] Beyond preserving the gut microbiota, intravesical antimicrobial therapy offers an additional potential advantage, i.e. the delivery of high concentrations of antimicrobials directly into the bladder. This targeted approach ensures that even pathogens with higher minimum inhibitory concentrations can be effectively eradicated.

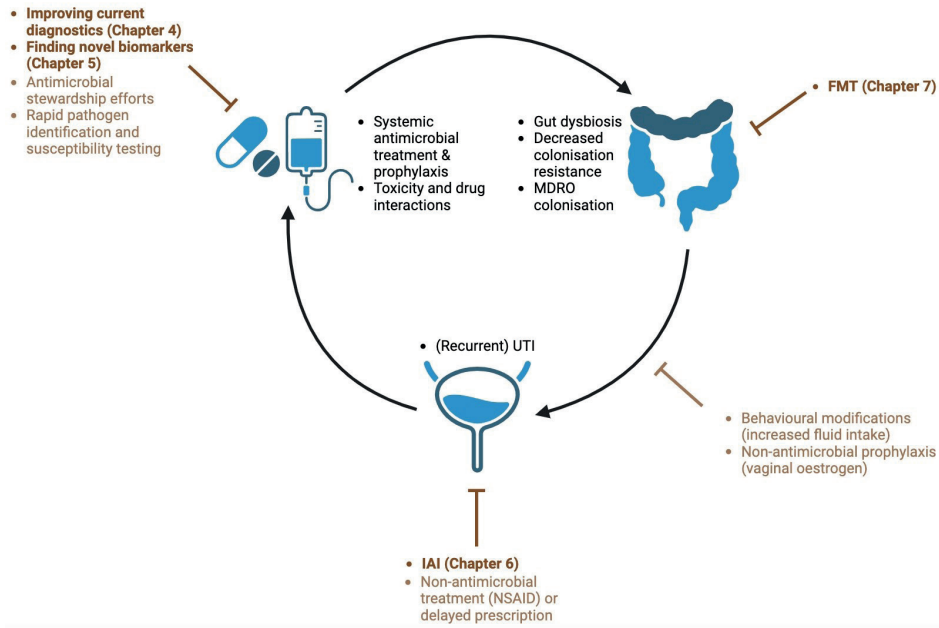


Figure 1: Vicious cycle of antimicrobial treatment and resistance. FMT = faecal microbiota transplantation, MDRO = multidrug resistant organism, IAI = intravesical aminoglycoside instillations, NSAID = non-steroidal anti-inflammatory drug, UTI = urinary tract infection

In **Chapter 6**, we describe the results of a cohort study including 44 patients who were treated with intravesical aminoglycoside instillations (IAI) in our institution. This study expands upon a prior study demonstrating efficacy of IAI in patients with rUTI, after which IAI was applied in a growing number of patients for longer durations. [30] Patients with multiple treatment cycles (on and off IAI) acted as their own controls. We found that IAI increased the time to the first recurrence and reduced the number of recurrences. This, together with the fact that one in four recurrences could be treated with daily instillations, reduced the number of oral and intravenous antimicrobial prescriptions. Moreover, serum aminoglycoside levels were undetectable in all but one patient, confirming the non-systemic potential of intravesical administration.

Furthermore, we found that the rate of UTI being caused by MDROs did not increase over the study period (18 and 14% off and on IAI respectively). We did see some instances of UTI due to aminoglycoside resistant Enterobacterales, but these

could be treated by oral antimicrobials and did not recur despite continuation of the same aminoglycoside. These aminoglycoside resistant strains are probably not the result of induced resistance, as IAI is gut-sparing, but rather the results of previous systemic antimicrobial treatment. In the previous study by Stalenhoef et al. [30] the rate of UTI being caused by MDRO dropped from 78 to 23%. We did not routinely perform faecal swabs in our patients, yet Stalenhoef et al. [30] found that intestinal colonisation remained relatively low at approximately 15%. Both Stalenhoef et al. and we did not investigate gut microbial richness, so whether IAI can alleviate gut dysbiosis remains an open question.

No malignancies were found on follow-up cystoscopy. While our data cannot definitively exclude the carcinogenic potential of long-term IAI, our relatively long follow-up period (more than 3.5 years for 25% of study participants) does diminish prior concerns.

Future studies should focus on the development of different antimicrobials for IAI. For instance, we observed more enterococcal infections in patients on IAI, which is likely explained by the fact that enterococci are frequently intrinsically resistant to high levels of aminoglycosides. Leaving aside the question of whether enterococcal infections should be treated at all, intravesical instillations with a vancomycin-containing regimen could address this matter. However, vancomycin does not have appropriate pharmacokinetic properties for intravesical installation as its efficacy is time-dependent rather than peak concentration-dependent, and intravesical antimicrobial concentrations rapidly decline due to urinary dilution and frequent voiding. In contrast with aminoglycosides, glycopeptides such as vancomycin do not exhibit a significant post-antibiotic effect.

Currently, methods to extend bladder incubation time of antimicrobials are being developed, for instance by means of nanoparticles. [31] These particles have been shown to promote endocytosis of antimicrobials into the urothelium in *in vitro* bladder models. Other non-antibiotic formulations are being investigated as well. The antiseptic cetylpyridinium chloride, which is a quaternary ammonium salt already being used in mouthwashes and eye drop formulations, was studied in three women with rUTI caused by extensively resistant uropathogens, with moderate success. [32] Other groups are exploring the potential of trimeric thiomannoside clusters, which prevent adherence of *E. coli* to the urothelium by inhibition of FimH adhesin. [31]

These new developments suggest that intravesical therapies are a promising modality for rUTI. However, the inconvenience of intravesical instillations will likely preclude its becoming a first-line modality. In our cohort, we found high treatment satisfaction scores. However, our population mainly consisted of postmenopausal women who had already failed continuous oral antimicrobial prophylaxis or who were immunocompromised due to kidney transplantation. As such, this group was highly motivated to try new therapies. For patients with a lower disease burden, intravesical installation, which requires clean intermittent catheterisation, might prove to be too invasive.

Another avenue to break the vicious cycle of gut dysbiosis and AMR, is by therapy directly targeting the gut. The most well-known example of effective gut restorative therapy is faecal microbiota transplantation (FMT) in patients with recurrent *Clostridioides difficile* infection (rCDI), a condition also characterised by gut dysbiosis. FMT involves introducing processed stool bacteria obtained from a healthy donor into the intestinal tract of a patient. In patients with rCDI, FMT has been shown to increase microbiota diversity and decrease the number of antibiotic resistance genes. [33, 34] Multiple studies have shown high cure rates in patients with rCDI, and FMT has become a widely used treatment modality for rCDI in clinical practice. [35]

In **Chapter 7**, we describe the results of a systematic review including recent studies that had investigated the efficacy of FMT for intestinal MDRO decolonisation. We found considerable heterogeneity between studies regarding the population, type of MDRO (mostly carbapenem-resistant Enterobacterales or vancomycin-resistant *Enterococcus*), route of administration, post-FMT antimicrobial use, and duration of follow-up. Although decolonisation rates varied greatly, the largest study showed significantly higher decolonisation rates in the FMT group compared with controls (66% versus 25% at 6 months, respectively). [36] Intriguingly, in two studies in the review, FMT showed a robust reduction in the number of MDRO infections (including UTI) even though the decolonisation rates were modest. [37, 38] A similar effect was seen in two other studies that were not included in the review. One study investigated the use of FMT in rCDI, and coincidentally found a reduced number of UTI recurrences (median 4 to 1 infections per year pre- and post-FMT, respectively). [39] While this finding might be explained by resolution of diarrhoea (thereby decreasing the risk of periurethral colonisation and exogeneous infection), it might also be explained by restoration of gut microbial richness and thus colonisation resistance. Recently, a small study showed FMT to be highly

effective in eradicating intestinal extended-spectrum beta-lactamase producing *E. coli* in kidney transplant recipients with rUTI. [40] In our tertiary care hospital, we are currently conducting a randomised clinical trial comparing FMT and oral decontamination with polymyxin and neomycin to oral decontamination only in the same target population, although its primary aim is to assess the safety of FMT in this population. Future studies should focus on the question whether FMT can not only reduce intestinal MDRO colonisation but also prevent recurrent infection in patients with rUTI.

The widespread application of FMT is impeded by the drawbacks inherent to this therapy: it is costly and invasive, and imposes a burden not only on the recipients but on donors as well. Alternatively, one could only administer selected components of the intestinal microbiota. For instance, a recent study showed that oral capsules composed of purified Firmicutes were highly effective in preventing a recurrent episode in patients with rCDI. [41]

Conclusion

Tackling the patient burden of UTI against the backdrop of increasing AMR will require a multifaceted approach, addressing both diagnostic and therapeutic knowledge gaps. To that end, this thesis underscores the importance of uniform research definitions and proposes a new reference standard. Furthermore, it shows the potential of both existing and new diagnostics for UTI in older patients, allowing for a more judicious use of antimicrobials. Finally, it highlights two alternative modalities for the management of patients with rUTI and MDRO colonisation. Follow up studies, building upon the work presented in this thesis, are already underway. As astutely put by Angela Huttner, UTI research is not an intellectual dead end, yet an exciting new frontier. [42]

References

1. Denkinger CM, Grant AD, Denkinger M, Gautam S, D'Agata EM. Increased multi-drug resistance among the elderly on admission to the hospital--a 12-year surveillance study. Arch Gerontol Geriatr 2013; 56(1): 227-30.
2. Miller LG, McKinnell JA, Singh RD, et al. Decolonization in Nursing Homes to Prevent Infection and Hospitalization. N Engl J Med 2023; 389(19): 1766-77.
3. Evaluation of medicinal products indicated for treatment of bacterial infections. Available at: https://www.ema.europa.eu/en/evaluation-medicinal-products-indicated-treatment-bacterial-infections Accessed 8th of June.
4. Uncomplicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/uncomplicated-urinary-tract-infections-developing-drugs-treatment-guidance-industry Accessed 8th of June.
5. Complicated Urinary Tract Infections: Developing Drugs for Treatment. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/complicated-urinary-tract-infections-developing-drugs-treatment Accessed 8th of June.
6. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) Events. Available at: https://www.cdc.gov/nhsn/psc/uti/. Accessed 8th of June.
7. Stone ND, Ashraf MS, Calder J, et al. Surveillance definitions of infections in long-term care facilities: revisiting the McGeer criteria. Infect Control Hosp Epidemiol 2012; 33(10): 965-77.
8. Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. J Clin Epidemiol 2014; 67(4): 401-9.
9. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis 2020; 71(6): 1367-76.
10. Wagenlehner FME, Cloutier DJ, Komirenko AS, et al. Once-Daily Plazomicin for Complicated Urinary Tract Infections. N Engl J Med 2019; 380(8): 729-40.
11. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 2013; 369(20): 1883-91.
12. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med 1982; 307(8): 463-8.
13. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46(12): 1813-21.
14. van den Broek D, Keularts IM, Wielders JP, Kraaijenhagen RJ. Benefits of the iQ200 automated urine microscopy analyser in routine urinalysis. Clin Chem Lab Med 2008; 46(11): 1635-40.

15. Schappert SM, Rechtsteiner EA. Ambulatory medical care utilization estimates for 2007. Vital Health Stat 13 2011; (169): 1-38.

16. Nicolle LE. Urinary Tract Infections in the Older Adult. Clin Geriatr Med 2016; 32(3): 523-38.

17. Nederlands Huisartsen Genootschap (NHG) standaard: urineweginfecties (Dutch primary care guideline: UTI). Available at: https://richtlijnen.nhg.org/standaarden/urineweginfecties. Accessed 15th of March 2023.

18. Dolscheid-Pommerich RC, Klarmann-Schulz U, Conrad R, Stoffel-Wagner B, Zur B. Evaluation of the appropriate time period between sampling and analyzing for automated urinalysis. Biochem Med (Zagreb) 2016; 26(1): 82-9.

19. Ching CB, Gupta S, Li B, et al. Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. Kidney Int 2018; 93(6): 1320-9.

20. Linder A, Soehnlein O, Akesson P. Roles of heparin-binding protein in bacterial infections. J Innate Immun 2010; 2(5): 431-8.

21. Shields-Cutler RR, Crowley JR, Miller CD, Stapleton AE, Cui W, Henderson JP. Human Metabolome-derived Cofactors Are Required for the Antibacterial Activity of Siderocalin in Urine. J Biol Chem 2016; 291(50): 25901-10.

22. Kjolvmark C, Tschernij E, Oberg J, Pahlman LI, Linder A, Akesson P. Distinguishing asymptomatic bacteriuria from urinary tract infection in the elderly - the use of urine levels of heparin-binding protein and interleukin-6. Diagn Microbiol Infect Dis 2016; 85(2): 243-8.

23. Rodhe N, Lofgren S, Strindhall J, Matussek A, Molstad S. Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria. Scand J Prim Health Care 2009; 27(2): 74-9.

24. Florkowski C, Don-Wauchope A, Gimenez N, Rodriguez-Capote K, Wils J, Zemlin A. Point-of-care testing (POCT) and evidence-based laboratory medicine (EBLM) - does it leverage any advantage in clinical decision making? Crit Rev Clin Lab Sci 2017; 54(7-8): 471-94.

25. Boon HA, De Burghgraeve T, Verbakel JY, Van den Bruel A. Point-of-care tests for pediatric urinary tract infections in general practice: a diagnostic accuracy study. Fam Pract 2022; 39(4): 616-22.

26. Bonkat G, Bartoletti F, Bruyere F, et al. EAU Guidelines on Urological Infections. Available at: https://uroweb.org/guidelines/urological-infections. Accessed 25th of February 2023.

27. Sorbara MT, Pamer EG. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. Mucosal Immunol 2019; 12(1): 1-9.

28. Worby CJ, Schreiber HLt, Straub TJ, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol 2022; 7(5): 630-9.

29. Sylvester RJ, van der Meijden AP, Witjes JA, Kurth K. Bacillus calmette-guerin versus chemotherapy for the intravesical treatment of patients with carcinoma in situ of the bladder: a meta-analysis of the published results of randomized clinical trials. J Urol 2005; 174(1): 86-91; discussion -2.

30. Stalenhoef JE, van Nieuwkoop C, Menken PH, Bernards ST, Elzevier HW, van Dissel JT. Intravesical Gentamicin Treatment for Recurrent Urinary Tract Infections Caused by Multidrug Resistant Bacteria. J Urol 2019; 201(3): 549-55.

31. Morris CJ, Rohn JL, Glickman S, Mansfield KJ. Effective Treatments of UTI-Is Intravesical Therapy the Future? Pathogens 2023; 12(3).

32. Zimmern PE, Sawant NV, Chang SS, Warner RW, De Nisco NJ. Intravesical VesiX as a Last Resort Therapy in Women With Antibiotic-Refractory Recurrent Urinary Tract Infections Contemplating Bladder Removal: A Preliminary Report. Ann Pharmacother 2023; 57(3): 350-1.

33. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. Clin Infect Dis 2016; 62(12): 1479-86.

34. Song Y, Garg S, Girotra M, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. PLoS One 2013; 8(11): e81330.

35. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013; 368(5): 407-15.

36. Seong H, Lee SK, Cheon JH, et al. Fecal Microbiota Transplantation for multidrug-resistant organism: Efficacy and Response prediction. J Infect 2020; 81(5): 719-25.

37. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. Clin Infect Dis 2021; 72(8): 1444-7.

38. Bar-Yoseph H, Carasso S, Shklar S, et al. Oral capsulized Fecal microbiota transplantation for eradication of carbapenemase-producing Enterobacteriaceae colonization with a metagenomic perspective. Clin Infect Dis 2020.

39. Tariq R, Tosh PK, Pardi DS, Khanna S. Reduction in urinary tract infections in patients treated with fecal microbiota transplantation for recurrent Clostridioides difficile infection. Eur J Clin Microbiol Infect Dis 2023; 42(8): 1037-41.

40. Woodworth MH, Conrad RE, Haldopoulos M, et al. Fecal microbiota transplantation promotes reduction of antimicrobial resistance by strain replacement. Sci Transl Med 2023; 15(720): eabo2750.

41. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. N Engl J Med 2022; 386(3): 220-9.

42. Huttner A. Urinary tract infection: from intellectual dead end to exciting new frontier? Clin Microbiol Infect 2023; 29(10): 1241.

Appendices

Nederlandse samenvatting

Dankwoord

Curriculum vitae

Publicatielijst

Nederlandse samenvatting

Deze samenvatting is geschreven om de inhoud van dit proefschrift toegankelijk te maken voor mensen zonder medische of academische achtergrond.

Urineweginfecties zijn infecties van de urinewegen: het gebied van de nieren, via de urineleiders (ureters) en de blaas tot de urinebuis (urethra). Bij mannen wordt ook de prostaat, die net onder de blaas gelegen is, tot de urinewegen gerekend.

Meestal ontstaan urineweginfecties doordat darmbacteriën zoals *Escherichia coli*, afgekort *E. coli*, van buiten de urethra binnendringen en zich verplaatsen naar de blaas. Daar hechten de bacteriën aan het blaasslijmvlies en kunnen ze een ontstekingsreactie uitlokken.

Er zijn heel veel verschillende soorten urineweginfecties. Sommige infecties beperken zich tot de blaas. Dit wordt een acute cystitis genoemd en gaat gepaard met pijn bij het plassen, frequenter plassen en loze aandrang. Bij mannen is doorgaans de prostaat ook betrokken en kan er naast de eerdergenoemde klachten ook koorts optreden. Maar er zijn ook ernstigere urineweginfecties. Bacteriën in de blaas kunnen zich ook via de urineleider naar de nieren verplaatsen. Dan ontstaat er een nierbekkenontsteking (acute pyelonefritis), die meestal gepaard gaat met koorts, koude rillingen en pijn in de zij. In 25–40% van de gevallen treedt er ook een bloedbaaninfectie (bacteriëmie) op. Dergelijke ernstigere urineweginfecties worden soms ook wel gecompliceerde urineweginfecties genoemd, in tegenstelling tot ongecompliceerde urineweginfecties zoals een acute cystitis.

Net zoals bij elke infectie spelen de witte bloedcellen (leukocyten) een belangrijke rol bij de afweer tegen urineweginfecties. Deze leukocyten komen ook in de urine terecht. De huidige diagnostiek van urineweginfecties berust op het aantonen van leukocyten in de urine. Daarnaast kan de urine gekweekt worden om de bacterie die de infectie veroorzaakt aan te tonen en te bepalen voor welke antibiotica die gevoelig is.

Urineweginfecties komen veel voor. In Nederland vinden jaarlijks meer dan twee miljoen bezoeken aan de huisarts plaats vanwege urineweginfecties, waarbij patiënten met koorts meestal worden verwezen naar de spoedeisende hulp. Acute cystitis en acute pyelonefritis komen meer voor bij vrouwen dan bij mannen, met een eerste piek op jongvolwassen leeftijd en een tweede piek na de overgang.

Bij jonge patiënten is de kans op een ernstig beloop laag, maar dat is anders bij oudere patiënten of patiënten bij wie de bacteriëmie leidt tot een septische shock (orgaanfalen door een overweldigende afweerreactie op een infectie). Van hen overlijdt ongeveer 30%.

De meest kwetsbare groep zijn vrouwelijke verpleeghuisbewoners, die meer kans hebben op urineweginfecties door bijkomende aandoeningen zoals verzakkingen, urine-incontinentie en een verzwakte afweer.

Voor zowel jonge als oudere vrouwen geldt dat de infectie in meer dan de helft van de gevallen terugkeert in het jaar na de eerste infectie. Zowel het frequent vóórkomen als terugkeren van urineweginfecties gaat gepaard met grote lijdensdruk voor de patiënten die het betreft en grote financiële kosten voor de maatschappij als geheel.

Daarnaast vormen urineweginfecties een belangrijke aanjager van antibiotica resistentie. Antibiotica zijn de hoeksteen van de behandeling van urineweginfecties, maar doordat ze zo veel en herhaaldelijk gebruikt worden, kunnen bacteriën resistent worden tegen de gegeven antibiotica.

Antibioticaresistentie is wereldwijd een groeiend probleem. Er zijn legio voorbeelden van bacteriën waar geen antibiotica in tabletvorm meer werkzaam tegen zijn. Deze infecties kunnen dus alleen met antibiotica via een infuus behandeld worden. Naarmate de resistentie toeneemt, moeten steeds zwaardere antibiotica gegeven worden, wat gepaard gaat met meer bijwerkingen. En inmiddels zijn er ook bacteriën die resistent zijn voor álle bestaande antibiotica, ook in infuusvorm. Voor patiënten die met zo'n bacterie geïnfecteerd zijn bestaat nu geen goede behandeling.

Deze bovenstaande punten worden uitgebreider behandeld in de inleiding van dit proefschrift in **hoofdstuk 1**.

De oplossing van het probleem van antibioticaresistentie en de lijdensdruk die gepaard gaat met (herhaaldelijke) urineweginfecties vraagt om meer onderzoek. Het probleem daarmee is echter dat er in de wetenschappelijke literatuur geen consensus is over hoe urineweginfecties gedefinieerd moeten worden. Er bestaan wel onderzoeksdefinities van Europese en Amerikaanse instanties, maar die zijn onderling verschillend.

In **hoofdstuk 2** wordt een overzicht gepresenteerd van 47 studies naar de behandeling van urineweginfecties die tussen 2019 en 2022 zijn verschenen, waarbij we hebben gekeken hoe urineweginfecties in die studies gedefinieerd werden. Bijna elke studie bleek zijn eigen criteria en afkapwaarden te gebruiken. De bestaande onderzoeksdefinities werden nauwelijks gevolgd. Het aantonen van leukocyten in de urine was bijvoorbeeld slechts in 28% van de studies vereist en de aanwezigheid van bacteriën in een urinekweek in 55% van de studies.

Ook werden de verschillende soorten urineweginfecties op verschillende wijzen gedefinieerd. Met name de term gecompliceerde urineweginfectie leidt tot veel onduidelijkheid: sommige studies gebruiken de term voor elke urineweginfectie die zich niet beperkt tot de blaas. Andere studies gebruiken de term juist voor een urineweginfectie in een patiënt met onderliggende aandoeningen, onder andere van de urinewegen, die de kans op een ernstig beloop vergroten. Door deze verschillen is het lastig om studies met elkaar te vergelijken of op waarde te schatten.

Daarom hebben wij een standaard opgesteld voor de definities van urineweginfecties voor onderzoeksdoeleinden. Deze standaard is tot stand gekomen met behulp van een groep van 57 internationale experts van verschillende medische specialismen door middel van een zogenaamde Delphi-methode. Bij deze methode wordt op een gestructureerde manier in meerdere rondes tot een consensus gekomen, waarbij gebruik is gemaakt van vragenlijsten en fictieve patiëntscenario's. Dit leidde tot het opstellen van een referentiestandaard met 94% consensus. Deze standaard en de Delphi-methode staan beschreven in **hoofdstuk 3**.

Onze referentiestandaard wijkt op belangrijke punten af van de eerder genoemde standaarden. Er zijn nieuwe afkapwaarden voor het aantal leukocyten en bacteriën in de urine opgesteld. Daarnaast wordt de mate van zekerheid over de diagnose meegenomen in de standaard: we onderscheiden mogelijke (possible), waarschijnlijke (probable) en zekere (definite) urineweginfecties. Hiermee brengen we de onderzoeksdefinities dichter bij de klinische praktijk, waar vaak ook sprake is van enige onzekerheid.

Verder spreken we niet meer van gecompliceerde urineweginfecties, maar wordt een onderscheid gemaakt tussen urineweginfecties met en zonder systemische verschijnselen (zoals koorts of lage bloeddruk). Dit helpt ook om het onderzoek, bijvoorbeeld naar nieuwe antibiotica, beter aan te laten sluiten op de praktijk.

Tenslotte heeft onze referentiestandaard, in tegenstelling tot de eerdere standaarden, specifiek aandacht voor ouderen. Ouderen worden buitensporig veel getroffen door urineweginfecties. Niet alleen komen urineweginfecties vaker voor bij ouderen, maar ze hebben vaak ook een ernstiger beloop. Ook zijn urineweginfecties bij ouderen moeilijker te diagnosticeren. Het uitvragen van klachten van oudere patiënten kan moeilijker zijn bij dementie of andere geheugenstoornissen. Daarnaast kunnen de klachten van een urineweginfectie lijken op klachten van andere aandoeningen die veel bij oudere vrouwen voorkomen, zoals verzakking of vaginale droogte. Tenslotte zijn de diagnostische testen, zoals het bepalen van leukocyten in de urine, bij ouderen minder betrouwbaar. Daarom heeft onze referentiestandaard andere afkapwaarden voor ouderen.

Minder betrouwbare diagnostiek is een probleem, omdat een behandelaar ten onrechte kan concluderen dat een patiënt een urineweginfectie heeft, terwijl dat in werkelijkheid niet zo is. In dat geval krijgt iemand ten onrechte antibiotica, die niet nodig zijn maar wel bijwerkingen kunnen veroorzaken. Ook dragen onterecht voorgeschreven antibiotica bij aan het wereldwijde probleem van antibioticaresistentie.

De aanwezigheid van leukocyten in de urine vormt bij ouderen een minder betrouwbare test voor het vaststellen van een urineweginfectie, omdat bij ouderen soms ook bacteriën en leukocyten in de urine aanwezig kunnen zijn zónder dat er sprake is van een urineweginfectie. Dit wordt asymptomatische bacteriurie genoemd en het komt veel voor. Van de oudere vrouwen die in een verpleeghuis wonen heeft de helft asymptomatische bacteriurie. Dit behoeft geen behandeling, maar is dus op basis van de aanwezigheid van leukocyten in de urine niet goed te onderscheiden van een urineweginfectie.

Daarom hebben we in **hoofdstuk 4** gekeken of het onderscheid tussen urineweginfecties en asymptomatische bacteriurie wel gemaakt kan worden door te kijken naar het precieze aantal leukocyten in de urine. Daarbij hebben we gebruik gemaakt van geavanceerde manieren om het aantal leukocyten exact te bepalen. In deze studie hebben we 164 vrouwen van 65 jaar en ouder met en zonder urineweginfecties onderzocht uit huisartsenpraktijken, verpleeghuizen en ziekenhuizen. Een deel van de vrouwen zonder urineweginfecties had asymptomatische bacteriurie. We vonden dat vrouwen met een urineweginfectie veel meer leukocyten in de urine hadden dan vrouwen met asymptomatische bacteriurie. Op basis daarvan konden we concluderen dat de meest gangbare

afkapwaarde voor leukocyten in de urine van 10 per microliter veel te laag is. Wij vonden een optimale afkapwaarde van 264 leukocyten per microliter. Als die wordt aangehouden, verbetert de betrouwbaarheid van de test en zullen minder vaak ten onrechte urineweginfecties worden vastgesteld. Daarmee wordt ook voorkomen dat de daadwerkelijke oorzaak van de klachten gemist wordt. Deze hogere afkapwaarde hebben we ook opgenomen in de referentiestandaard van hoofdstuk 3.

Daarnaast hebben we in dezelfde groep vrouwen onderzocht of er andere mogelijke testen zijn voor het diagnosticeren van urineweginfecties. De resultaten van dat onderzoek staan beschreven in **hoofdstuk 5**. In dit onderzoek hebben we naar twaalf verschillende biomarkers in de urine gekeken. Dat zijn in het lichaam geproduceerde stoffen die gerelateerd zijn aan ontstekingsreacties en schade aan de urinewegen. Vijf daarvan konden goed het onderscheid maken tussen wel of geen urineweginfectie. Bij vrouwen met asymptomatische bacteriurie, bij wie het onderscheid het lastigste te maken is, bleken deze vijf biomarkers ook van aanvullende waarde bovenop het leukocytenaantal. Op basis van deze resultaten is nu een vervolgstudie opgezet in een diversere groep patiënten. Het doel is om deze biomarkers verder te ontwikkelen tot nieuwe testen die nog nauwkeuriger urineweginfecties kunnen diagnosticeren.

Betere diagnostiek van urineweginfecties kan helpen om onterechte behandelingen met antibiotica te voorkomen, wat tot minder bijwerkingen en minder antibioticaresistentie kan leiden. Maar dit helpt alleen mensen die geen urineweginfectie hebben. Patiënten mét een urineweginfectie hebben de antibiotica uiteraard wel degelijk nodig.

Daar komt bij dat urineweginfecties vaak terugkomen. Als er sprake is van drie of meer urineweginfecties per jaar, spreekt men van recidiverende urineweginfecties. Zoals eerder genoemd, gaan die gepaard met grote kosten en een verminderde kwaliteit van leven en dragen ze in belangrijke mate bij aan antibioticaresistentie.

Patiënten met recidiverende urineweginfecties krijgen soms een onderhoudsbehandeling met antibiotica om nieuwe infecties te voorkomen. Dit lukt helaas niet altijd, bijvoorbeeld doordat de patiënt ernstige bijwerkingen heeft of drager is van resistente bacteriën, en er geen antibiotica in tabletvorm meer gegeven kunnen worden. In het Leids Universitair Medisch Centrum worden dergelijke patiënten sinds meer dan tien jaar behandeld met blaasspoelingen met antibiotica. Hiervoor wordt via eenmalige urinekatheters een vloeistof met antibiotica in de

blaas achtergelaten. Patiënten worden hierin getraind zodat zij dit zelf thuis kunnen doen. Een voordeel van deze methode is dat de antibiotica alleen in de blaas werken en daardoor in theorie veel minder bijwerkingen veroorzaken dan antibiotica die systemisch (d.w.z. in het hele lichaam) werken.

Hoewel deze behandeling al geruime tijd wordt toegepast, is er nog maar weinig bekend over de effectiviteit en veiligheid op lange termijn. In **hoofdstuk 6** beschrijven wij onze ervaringen met deze blaasspoelingen. Vierenveertig patiënten die tussen 2013 en 2022 zijn behandeld zijn meegenomen in deze studie. Onder blaasspoelingen was het aantal recidief urineweginfecties 25% lager en waren er veel minder vaak systemische antibiotica nodig. Patiënten waren over het algemeen erg tevreden over de behandeling. Er werden geen complicaties en geen gevallen van blaaskanker gezien. Blaasspoelingen met antibiotica blijken dus veilig en effectief. Doordat ze alleen in de blaas werken en niet in de rest van het lichaam, hebben ze ook geen negatieve invloed op de darmflora, zoals systemisch werkende antibiotica dat wel hebben.

Het is bekend dat vrouwen met recidiverende urineweginfecties een slechtere kwaliteit darmflora hebben, waarschijnlijk veroorzaakt door de vele antibiotische behandelingen. Een slechte darmflora vergroot de kans op het ontstaan van multiresistente darmbacteriën: bacteriën die resistent zijn tegen meerdere soorten antibiotica. Patiënten die drager zijn van multiresistente bacteriën in hun darmflora hebben een grotere kans op ernstige infecties en overlijden. Als een dergelijke ernstige infectie optreedt, wordt deze opnieuw behandeld met antibiotica, wat weer bijdraagt aan een verdere verslechtering van de darmflora. Maar het is niet makkelijk om deze vicieuze cirkel te doorbreken en de darmflora te verbeteren. Eerdere studies hebben laten zien dat lokaal werkende antibiotica in de darm niet effectief zijn en het probleem mogelijk zelfs verergeren.

Een mogelijke behandeling is het vervangen van de darmflora door middel van een fecestransplantatie. Hierbij wordt de ontlasting van gezonde vrijwilligers gebruikt. Er is vooral ervaring met fecestransplantaties in de behandeling van darminfecties veroorzaakt door de bacterie *Clostridioides difficile*, maar nog niet zo veel bij het behandelen van dragerschap van multiresistente bacteriën. Dat laatste wordt behandeld in **hoofdstuk 7**. Dit is een overzicht van recente studies die gedaan zijn naar fecestransplantaties als behandeling van dragerschap. Wij vonden in de literatuur zeven kleine niet-gerandomiseerde studies en vijf patiëntbeschrijvingen, gepubliceerd sinds 2020. Na fecestransplantatie loste in 20 tot 90% van de gevallen

het dragerschap voor de multiresistente bacteriën op. Hoewel fecestransplantatie niet altijd het dragerschap oploste, werden er in sommige onderzoeken toch minder infecties met multiresistente bacteriën en minder urineweginfecties gezien. Mogelijk is een lichte verbetering in de darmflora al genoeg om deze patiënten tegen een infectie te beschermen. Om de precieze effectiviteit van fecestransplantaties vast te stellen, zijn betere onderzoeken nodig.

Hoofdstuk 8 tenslotte bevat de algemene discussie van het proefschrift, waarin alle bovenstaande onderwerpen besproken worden en vooruitgekeken wordt naar toekomstige toepassingen en ontwikkelingen. Zo is de voorgestelde referentiestandaard van hoofdstuk 3 goed ontvangen door de onderzoekers binnen het gebied van de urineweginfecties, maar de tijd zal het leren of de referentiestandaard ook echt gevolgd gaat worden in toekomstige studies. We hebben aanleiding om te denken dat dat inderdaad het geval zal zijn, omdat onze referentiestandaard breder toepasbaar en meer up-to-date is dan de eerder genoemde onderzoeksdefinities. Verder dient onze standaard uiteraard geüpdatet te worden als er nieuwe diagnostische tests worden ontwikkeld.

Daarvoor hebben we al een eerste aanzet gedaan met het identificeren van vijf nieuwe biomarkers. Voordat deze in de dagelijkse praktijk gebruikt kunnen worden, is niet alleen meer onderzoek nodig in andere groepen patiënten, maar ook zullen deze biomarkers ontwikkeld moeten worden tot praktisch uitvoerbare testen. In ons onderzoek hebben we geavanceerde laboratoriumonderzoeken gebruikt om deze biomarkers te bepalen, maar dit is duur en tijdrovend en de apparaten die hiervoor nodig zijn, staan alleen in (grotere) ziekenhuizen. De meeste urineweginfecties worden juist gediagnosticeerd bij huisartsen en in verpleeghuizen. Er moeten dus eenvoudigere tests ontwikkeld worden, die gemakkelijk en snel kunnen worden uitgevoerd zonder dat er een laboratorium aan te pas komt. Voor het aantonen van leukocyten in de urine bestaan zulke tests al: de dipstick die de huisarts gebruikt om urineweginfecties vast te stellen. Deze dipstick is echter minder betrouwbaar, zoals we hebben aangetoond in hoofdstuk 4. Dat is dus ook iets om rekening mee te houden als de vijf nieuwe biomarkers die we gevonden hebben in de vorm van een dipstick ontwikkeld worden.

Tenslotte zijn in dit proefschrift behandelingen besproken voor recidiverende urineweginfecties en dragerschap van multiresistente bacteriën. Voor fecestransplantatie is nog niet aangetoond dat het herstellen van de darmflora ook leidt tot minder urineweginfecties, dus dit is iets waar verder onderzoek

naar gedaan moet worden. Voor zowel blaasspoelingen als fecestransplantaties geldt dat het belastende behandelingen zijn, wat een bredere toepassing van deze methoden in de weg staat. Hiervoor dienen eenvoudigere en minder ingrijpende toedieningswijzen ontwikkeld te worden.

Een deel van al deze ontwikkelingen en vervolgonderzoeken is al onderweg, waarvan we de komende jaren de resultaten zullen gaan zien. Alleen door innovaties in diagnostische testen en behandelmethoden voor urineweginfecties kan de ziektelast voor individuele patiënten worden verminderd en kan het gevaar van antibioticaresistentie voor onze samenleving worden ingeperkt.

Dankwoord

Allereerst wil ik de vrouwen bedanken die hebben deelgenomen aan de onderzoeken beschreven in hoofdstuk 4 en 5 van dit proefschrift. Jullie bijdrage is van onschatbare waarde, ook voor jongere generaties vrouwen.

Leo, het was een voorrecht om jou als mijn promotor en mentor te hebben, omdat ik altijd bij je naar binnen kon lopen en je mijn vertrouwen als onderzoeker enorm hebt versterkt. Je inspireerde mij met ideeën die ik niet zelf had bedacht, maar die toch resoneerden als mijn eigen ontdekkingen.

Merel, ondanks dat ik één van je eerste promovendi was, wist ik vanaf het begin dat het goed zat, door jouw ongeëvenaarde drive en vastberadenheid. Daar waar ik obstakels zag, kwam jij altijd met briljante oplossingen, zelfs als je op de skipiste stond.

Simon, we've only ever met in person once, and even though most of our meetings were through Teams, I always caught myself smiling after our meetings. Your humour and attention for the person behind the PhD student have made a lasting impression.

Sandra, dankzij jou wist ik tijdens mijn bachelor al dat ik infectioloog wil worden. Jij hebt me als eerste laten zien dat onderzoek leuk is en ik wil je oprecht bedanken dat je mij in verbinding hebt gebracht met Merel.

I would like to thank Caroline, Tamara, Cees, Lona, Jeffrey, Suzanne, Béla, and Florian for our fruitful discussions: it was wonderful to spend so much time with a group of UTI enthusiasts.

Rozemarijn, ik wil je bedanken voor het vele werk dat je hebt verricht voor de systematic review.

Esther, bedankt voor je inzet bij de SENIOR-studie en daarna. Ik heb dankbaar gebruik gemaakt van je flexibiliteit en je inhoudelijke kennis als ik weer eens iets wilde veranderen aan het protocol.

Rolf, bedankt voor je hulp bij de fijnere punten van de epidemiologie.

Marjan, door jou is de SENIOR-studie drie keer zo snel aan zijn inclusiedoel gekomen. Ik vind het nog steeds moeilijk om dingen te delegeren, maar bij jou had ik daar nooit last van. Bedankt voor alles.

Liesbeth, ik gun iedere promovendus een Liesbeth. Jouw luisterende oor en jouw positiviteit waardeer ik enorm.

Alle huisartsenpraktijken, verpleeghuizen en ziekenhuizen die deelnemers hebben geïncludeerd voor de SENIOR-studie wil ik bedanken voor hun inzet. In het bijzonder wil ik Lenneke noemen. Je deed het met grote vanzelfsprekendheid, maar verzette ondertussen bergen. Ook Brenda wil ik heel erg bedanken voor alle inspanningen.

Ik wil alle internisten uit het HMC en LUMC bedanken voor hun begeleiding en mijn vorming tot internist. In het bijzonder wil ik Luc noemen: ik heb genoten van al onze niet-wetenschappelijke gesprekken.

Maaike en Annette, het lot heeft beschikt dat wij met zijn drieën op één kamer (zonder ramen) aan onze promotietrajecten mochten werken. Bijna alle diepe dalen en hilarische momenten (niet gepast voor een dankwoord) speelden zich af binnen de vier muren van ons zweetkamertje. Ook onze wereldreizen waren episch, of we nu vanuit een hotelbed in Lissabon online het congres volgden of in New York noodgedwongen nóg closer werden. Meiden, we made it.

Rowan, we begrijpen elkaars grappen al voordat ze zijn uitgesproken. Mede daarom ben je een van mijn beste vrienden. Laten we snel afspreken op Vlieland. Of Ameland.

In alfabetische volgorde, omdat ik iedereen evenveel waardeer: Bram, Connie, David, Dook, Ernst-Jan, Esmée, Isabelle, Jesse, Josephine, Lorena, Marinus, Nadim, Neeltje, Niels, Noah, Patricia, Pim, Thomas, Tim, Tineke en Wing-Yi, bedankt voor jullie vriendschap en dat jullie me eraan herinneren dat het leven niet alleen draait om promoveren.

Toon, Jeanne, Roel, Sandra, Krijn, Dominique, Janneke en Max, bedankt dat ik me vanaf het eerste moment lid van de familie voelde. Jullie hebben een echte carnavaller van me gemaakt. Nova en Mika, bedankt voor jullie steun en geduld tijdens deze reis.

Pap, ik ben dankbaar voor wat ik van je heb geleerd en bij het schrijven van dit boekje heb ik vaak gedacht aan hoe we vroeger samen teksten schreven, onder andere voor mijn (gefaalde) zangcarrière. Ik koester onze hechte band.

Mam, jouw onvoorwaardelijke liefde heeft mij door moeilijke momenten gedragen en mijn successen verrijkt. Ik ben je eeuwig dankbaar voor jouw grenzeloze steun.

Camiel, de band die wij hebben is niet in woorden onder te brengen. Dat hoeft ook niet, omdat jij en ik aan een blik al genoeg hebben. Broertje, deze achtbaan is af.

Bram, zonder jou was dit boekje er niet geweest. Dat zul je uiteraard ontkennen, maar je moet het maar van me aannemen. Ik heb onze wedijver over wie het eerste klaar zou zijn verloren, maar ik heb met jou als partner iets veel belangrijkers gewonnen. *Jag älskar dig.*

Curriculum vitae

Manu Bilsen is op 11 augustus 1993 geboren in Amsterdam. Samen met zijn tweelingbroer Camiel groeide hij op in Bergen. In 2011 behaalde hij zijn Europees Baccalaureaat *summa cum laude* aan de Europese School in Bergen. Hierna startte hij met de studie geneeskunde aan de Universiteit Leiden. Zijn masterscriptie (onder supervisie van dr. Mariëtte Boon en prof dr. Patrick Rensen) werd genomineerd voor de 'LUMC student research award', toegekend aan de beste masterscriptie. Tijdens verdiepende stages bij de interne geneeskunde in het Bronovo ziekenhuis en de infectieziekten in het LUMC, ontwikkelde hij een passie voor infectieziekten. In 2017 rondde hij *cum laude* de master geneeskunde af, waarna hij klinische ervaring opdeed als ANIOS interne geneeskunde in het Haaglanden Medisch Centrum. Een jaar later startte hij met de specialisatie tot internist in datzelfde ziekenhuis (opleider dr. Aart Bootsma). In 2021 onderbrak hij de opleiding voor een promotietraject onder leiding van dr. Merel Lambregts en prof. dr. Leo Visser in het LUMC en prof. dr. Simon Conroy aan University College London. Tijdens zijn promotietraject bezocht hij congressen in onder andere Lissabon, Kopenhagen en Washington D.C. en won hij in 2022 de prijs voor de beste abstractpresentatie op de internistendagen. In 2023 vervolgde hij de opleiding tot internist in het LUMC (opleiders dr. Natasha Appelman-Dijkstra, prof. dr. Hans de Fijter en prof dr. Leo Visser) en zijn differentiatie tot internist-infectioloog in 2024 (opleider dr. Sandra Arend). Samen met zijn vriend Bram woont hij in Utrecht.

Publicatielijst

1. **Bilsen MP**, Conroy SP, Schneeberger C, Platteel TN, van Nieuwkoop C, Mody L, et al. A reference standard for urinary tract infection research: a multidisciplinary Delphi consensus study. Lancet Infect Dis. 2024.
2. **Bilsen MP**, Treep MM, Aantjes MJ, van Andel E, Stalenhoef JE, van Nieuwkoop C, et al. Diagnostic accuracy of urine biomarkers for urinary tract infection in older women: a case-control study. Clin Microbiol Infect. 2024;30(2):216-22.
3. **Bilsen MP**, Aantjes MJ, van Andel E, Stalenhoef JE, van Nieuwkoop C, Leyten EMS, et al. Current Pyuria Cutoffs Promote Inappropriate Urinary Tract Infection Diagnosis in Older Women. Clin Infect Dis. 2023;76(12):2070-6.
4. **Bilsen MP**, Lambregts M, Conroy S. Guideline commentary on updated NICE guidelines for urinary tract infections. Age Ageing. 2023;52(3).
5. **Bilsen MP**, van Uhm JIM, Stalenhoef JE, van Nieuwkoop C, Groenwold RHH, Visser LG, et al. Intravesical aminoglycoside instillations as prophylaxis for recurrent urinary tract infection: patient satisfaction, long-term safety and efficacy. JAC Antimicrob Resist. 2023;5(2):dlad040.
6. **Bilsen MP**, Jongeneel RMH, Schneeberger C, Platteel TN, van Nieuwkoop C, Mody L, et al. Definitions of Urinary Tract Infection in Current Research: A Systematic Review. Open Forum Infect Dis. 2023;10(7):ofad332.
7. **Bilsen MP**, Lambregts MMC, van Prehn J, Kuijper EJ. Faecal microbiota replacement to eradicate antimicrobial resistant bacteria in the intestinal tract - a systematic review. Curr Opin Gastroenterol. 2022;38(1):15-25.
8. Nahon KJ, Janssen LGM, Sardjoe Mishre ASD, **Bilsen MP**, van der Eijk JA, Botani K, et al. The effect of mirabegron on energy expenditure and brown adipose tissue in healthy lean South Asian and Europid men. Diabetes Obes Metab. 2020;22(11):2032-44.
9. **Bilsen MP**, van Meijgaarden KE, de Jong HK, Joosten SA, Prins C, Kroft LJM, et al. A novel view on the pathogenesis of complications after intravesical BCG for bladder cancer. Int J Infect Dis. 2018;72:63-8.
10. van Grootveld R, **Bilsen MP**, Boelsums TL, Heddema ER, Groeneveld GH, Gooskens J, et al. Chlamydia caviae causing community-acquired pneumonia: an emerging zoonosis. Vector Borne Zoonotic Dis. 2018;18(11):635-7.