Epidemiology of Clostridium difficile infections in the Netherlands and Europe: implications for surveillance and control

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To all hospital
cleaning personnel

Chapter 1

Introduction

Clostridium difficile infection (CDI) was discovered in 1978 as an important cause of antibiotic-associated diarrhoea and pseudomembranous colitis [1]. CDI became the most common healthcare-associated infection in Northern-America and Europe during the antibiotic era, especially after global spread of a fluoroquinolone-resistant ribotype 027 strain originating from the Canadian province Quebec in 2003 [2, 3]. The rise of CDI in Northern-America and Europe urged the use of epidemiological surveillance systems to monitor disease dynamics and rapidly detect outbreaks [3]. In Europe, national surveillance activities were supported by the European Centre for Disease Prevention and Control (ECDC) gradually moving towards standardised epidemiological surveillance with molecular typing systems for CDI [4]. However, valid estimations of the infection burden of CDI in Europe were hampered by the heterogeneity and insufficiency of diagnostic algorithms for CDI, lack of standardised typing systems and incomplete surveillance methodologies. This thesis includes two studies conducted within a project named 'the European CDI Surveillance Network' (ECDIS-Net) focussing on enhancement of CDI surveillance and laboratory capacity for CDI in Europe. In the Netherlands, a sentinel epidemiological surveillance system monitors the incidence of CDI and the occurrence of outbreaks in hospitals, but not in other healthcare facilities or in the community. This thesis describes (spatial) trends in the epidemiology of CDI in the Netherlands according to sentinel surveillance, in particular for children and the potentially zoonotic *C. difficile* ribotype 078. Data of a community-based case-control study to estimate the burden of CDI in the community, was used to apply spatial scan statistics to detect CDI clustering beyond the hospital setting. Finally, this thesis provides directions for future epidemiological surveillance systems of CDI, both in the Netherlands and Europe.

Clostridium difficile

COMMENSAL AND PATHOGEN

Clostridium difficile is a ubiquitous gram-positive, spore-forming, rod-shaped bacterium, firstly observed in the bacterial flora of meconium from healthy neonates in 1935 by Hall and O'Toole [5]. The bacterium was called "*Bacillus difficile*" for its difficulty to be cultured, growing exclusively under anaerobic conditions. The bacterium did not cause any symptoms in the neonates studied, but appeared to be highly pathogenic in animal models due to production of an exotoxin [5]. It was not just a matter of chance that *C. difficile* was firstly isolated from neonates; *C. difficile* colonisation is the highest during early life in humans (prevalence of 26%, with a range of 18-90%) [6, 7].

C. difficile did not appear to be an important cause of disease before the antibiotic era [8 – 11]. The introduction of antibiotics, especially clindamycin in the 1960s, led to an increase of antibiotic-associated colitis [11 – 13]. In 1978, Bartlett et al. recognised toxins of *C. difficile* to be the cause of antibiotic-associated pseudomembraneous colitis in adults [1]. The bacterium was renamed "*Clostridium difficile*" (κλωστήρ: Greek for rod) [14]. Recently, the bacterium has been reclassified as *Clostridioides difficile* [15]. Subsequent studies confirmed antibiotic use to be the foremost risk-factor for *C. difficile* infection (CDI) [11]. Antibiotics create a niche for *C. difficile* growth and toxin production in the gut due to loss of other microbial communities that compete for nutrients and/or interfere with more specific metabolic pathways (e.g. bile acid metabolism) [16, 17]. During or soon after antibiotic use, the odds for getting CDI increase a 7–10-fold, and gradually normalise during a period of 3 months [18, 19]. The risk is dose dependent [20] for and the highest for (third-generation) cephalosporins and clindamycin [21, 22]. Disruption of the gut microbiota is not the exclusive preserve of antibiotics; also other drugs, such as proton pump inhibitors, affect the gut microbiota and increase the risk for CDI [23].

C. difficile belongs to the Peptostreptococcaceae family [15, 24] within the genus of Clostridia with more than 150 species. Around 15 of these species, including *C. difficile*, *C. perfringens*, and *C. tetani*, are capable to produce toxins, and thereby cause infection in humans [14]. *C. difficile* is known for its potential to produce a toxin A (tcdA), a toxin B (tcdB), and a binary toxin (*C. difficile* transferase; CDT) [25]. Some *C. difficile* subspecies carry one or more toxin genes ('*toxigenic*' strains) and these toxin genes can be acquired and lost though time [26]. Toxin A and B inactivate regulatory proteins (Rho-GTPases) of the cytoskeleton, and thereby cause apoptosis of the epithelial cells in the gut [27, 28]. Binary toxin is a ADP-ribosylating toxin that induces formation of microtubule-based protrusions that might facilitate adherence and colonisation [29, 30]. Its role in *C. difficile* virulence is a topic of debate [31]. *C. difficile* contains many other factors to invade and survive hostile circumstances [32]. For example, specific adhesion molecules on the cell surface of *C. difficile* were found to facilitate the binding and release from target cells [33]. The ecological fitness of *C. difficile* –linked to its ability to cause disease

and emerge– varies per genotype [34]). The exceptional ecological fitness of a genotype named PCR ribotype 027/NAP1 facilitated its worldwide transmission since 2003 and ability to cause outbreaks [2, 34].

Like other clostridia, *C. difficile* sporulates to survive the aerobic conditions outside the hosts gut, indispensable for transmission to other hosts (Figure 1) [35]. *C. difficile* spores are metabolically dormant and have a spore coat covered by an exosporium, causing resistance to other environmental stress-factors (e.g. antiseptics) as well [35, 36]. Besides, sporulation helps the bacterium to resist antibiotics and the host immune system after digestion [35]. *C. difficile* spores are excreted by both symptomatic as asymptomatic humans and animals [37, 38]. Yet, symptomatic patients have higher levels of skin and environmental contamination than asymptomatic carriers (Risk Difference 20%) [39].

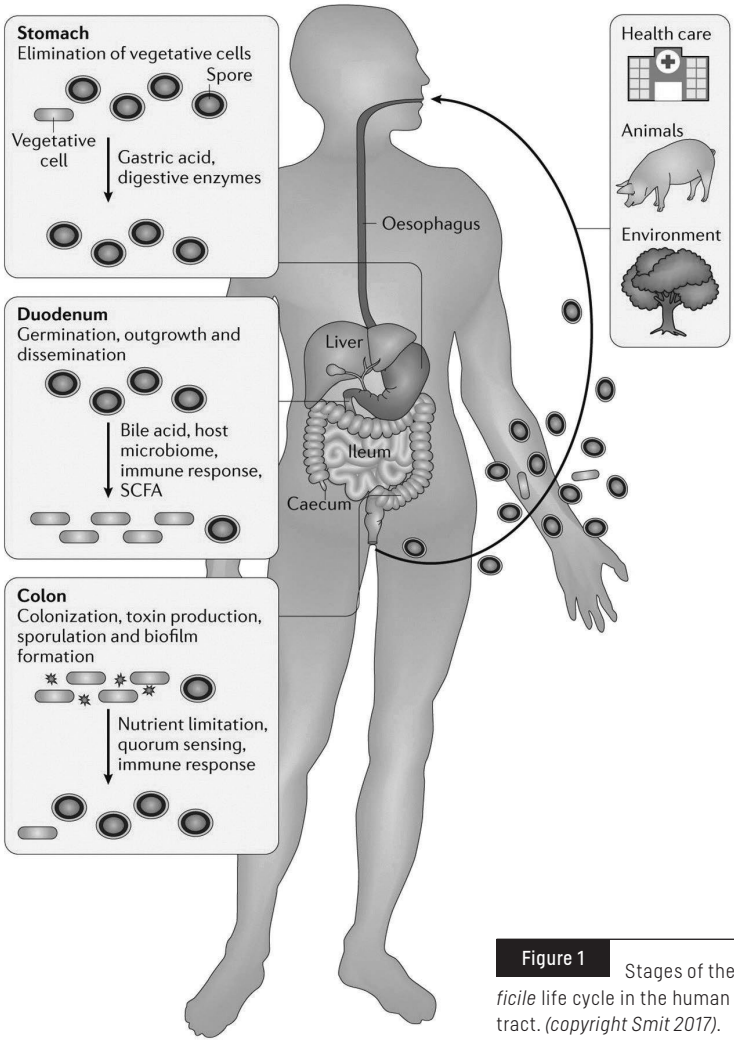

Figure 1 Stages of the *Clostridium difficile* life cycle in the human gastrointestinal tract. *(copyright Smit 2017).*

PREVENTION OF CDI

The presence of toxigenic *C. difficile* in the gut does not lead to infection in hosts with an adequate colonisation resistance and may even protect against CDI (Risk Ratio 0.3) [40, 41]. CDI occurs when growth and toxin production of commensal or recently acquired *C. difficile* surpasses the host resistance. The host resistance is influenced antibiotic use, age, underlying diseases and other drugs that affect the gut microbiota or immune system; the most well-described risk factors for CDI [42]. In hospitalised patients whom usually have a reduced colonisation resistance for CDI, exposure to symptomatic CDI patients and/or environmental contamination increases the risk for CDI (Hazard Rate 1.2) [43, 44]. Environmental *C. difficile* exposure in hospitals can be significantly reduced by daily and/or terminal bleach disinfection, and multifaceted campaigns to improve hand hygiene [45]. Restrictive antibiotic stewardship programmes (aiming to persevere the patient resistance for CDI) can reduce the incidence of CDI by 48-49%, although the evidence for this finding is weak [46, 47]. The largest preventative effects has been shown for daily and terminal bleach disinfection, antibiotic stewardship, and bundled interventions (e.g. improved hand hygiene, contact precautions, environmental cleaning, and antibiotic stewardship) [45, 48]. During an outbreak, these measures are usually intensified [49]. Recent developments in the field of high resolution molecular typing methods (whole-genome sequencing) resulted in a shift of previously generally accepted hypothesis on nosocomial CDI transmission. It was found that only 20-45% of hospital CDI results from direct transmission from other symptomatic patients [50 – 52]. Alternative reservoirs –within and beyond healthcare facilities– were not elucidated and need further investigation. Reservoirs include '*one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the target population*' [53]. Reservoir populations of *C. difficile* include several animal species (e.g. pets) and asymptomatic infant and adult carriers [54]. Besides, the living environment is widely contaminated with *C. difficile* spores; 25% of the parks, 17% of the households and 17% of the hospitals contains spores of toxigenic *C. difficile* (in southern United States) [55]. In addition, *C. difficile* spores are isolated from water and food in varying percentages [36, 37]. Therefore, tracing the attribution of different *C. difficile* sources to CDI is extremely complex but would be useful to reduce exposure, especially in those with a decrease of colonisation resistance. Antibiotic stewardship in the outpatient setting is important as in hospitals; e.g. a 10% reduction of antibiotic use could prevent 17% of the community-acquired CDI cases [56].

DIAGNOSING CDI

The range of symptoms caused by toxigenic *C. difficile* is wide and overlaps other gastrointestinal infections. Severe damage of the colon by *C. difficile* toxins results in elevated yellow-white nodules or plaques consisting of neutrophils, nuclear debris and other inflammatory elements on the mucosal surface ("pseudomem-

branes") observed by endoscopy [57] potentially leading to ileus, toxic megacolon, septic shock and death. The majority of the outpatients diagnosed with CDI has persistent diarrhoea, watery stools, weight loss, abdominal pain, and watery or slimy stools [58 – 60]. About 4-8% of the outpatients are hospitalised because of CDI and death due to CDI is rare (<1%) [58, 59, 61]. In contrast, hospital patients with CDI more often have systemic signs of infection indicative of severe disease [62] such as leucocytosis (29-50%) and fever (37-56%) [63, 64]. Within 30 days, 13% of the hospital patients with CDI decease, and 10% of these deaths were found to be related to CDI [65]. Children tend to have milder disease compared to adults [66]. Laboratory diagnosis of CDI is one of the most discussed topics in CDI surveillance. The main problem is the absence of a single diagnostic test that has optimal test characteristics (high sensitivity and specificity) and is easy and quick to perform. Besides, research and communication on CDI diagnostics is complicated by the presence of two golden standards; a cell cytotoxicity assay detecting free toxin in faeces and a cytotoxigenic culture detecting toxigenic *C. difficile*. In 2009, a two-step laboratory algorithms was recommended for diagnosis of CDI by the ESCMID diagnostic guideline for CDI [67]. Moreover, the guideline endorsed CDI laboratory testing of merely unformed stool samples using the '3-day rule' (testing samples of all diarrhoeic patients ≥72 hrs admitted to a healthcare facility). A revised ESCMID diagnostic guideline was published in 2016, e.g. abandoning use of toxin EIAs (without detection of GDH) as a first step of the diagnostic algorithm for CDI and adding an optional third step for indistinct cases (CDI or carriage of toxigenic *C. difficile*) [68].

CDI TREATMENT

CDI can resolve by discontinuation of the inciting antibiotic. When specific treatment is indicated, it is recommended to treat a first CDI with an oral ten-day course of metronidazole in mild cases, or a ten-day course of vancomycin in severe cases [69 – 71]. Metronidazole produces free radicals that break DNA strands and cause cell death of several anaerobic bacteria [72], whereas vancomycin inhibits cell-wall synthesis in gram-positives by binding to the peptidoglycan precursor [73]. Vancomycin has a higher symptomatic cure rate than metronidazole (79 vs. 72%; Risk Difference 7%) but is more expensive [74] and has more profound negative effects on the microbiota [75]. *C. difficile* resistance to both antibiotics is rare [76], but needs further clinical evaluation. Nine other antibiotics are available for CDI [74], among them fidaxomycin that has a lower recurrence rate compared to vancomycin in cases other than *C. difficile* ribotype 027 [69, 77] and mainly has its place for treatment of recurrent CDI [69]. Decision-making (supported by prediction models), involves consideration of disease severity, disease recurrence, causative factors, feasibility of oral treatment, *C. difficile* type and antibiotic susceptibility [69].

The antithesis of treating an antibiotic-induced disease with antibiotics [78] stimulated the development of new CDI treatments. The most striking 'novel' treatment

of CDI includes transfer of a healthy donors stool sample into the gut of an infected patient (faecal microbiota transfer; FMT) effective in 94% of the patients with recurrent CDI [79]. In 2015, a National Donor Feces Bank (NDFB) was initiated to provide FMT for Dutch hospitals in a standardised manner [80]. Alternative preventative or treatment modalities in development that focus on the host resistance against CDI are vaccination, human monoclonal immunotherapy [81], toxin binders, administration of non-toxigenic *C. difficile* strains [82] and microbiome-based strategies (e.g. administration of *Firmicutes* spores) [83, 84].

Epidemiological surveillance systems

AIMS AND METHODOLOGIES

Epidemiological surveillance systems originate from the mid-20th century and involve "*the systematic collection, analysis, interpretation and timely dissemination of health data for the planning, implementation and evaluation of public health programmes*" [85, 86]. Surveillance systems aim to generate "*a public health response leading to the control and prevention of adverse health events, or to a better understanding of the process leading to an adverse outcome*" (e.g. disease risk factors or transmission) [86]. Epidemiological surveillance systems are categorised into disease-specific, syndromic, and event-based surveillance [87]. Sub classifications refer to the method of data collection, e.g. differentiating healthcare provider-based from laboratory-based surveillance, active from passive surveillance, and prospective from retrospective surveillance [88]. Yet, 'retrospective surveillance' seems like a contradiction-in-terms that should be avoided. Another sub classification differentiates sentinel from national surveillance [89]. Sentinel surveillance refers to collection of data from a representative fraction of the population to monitor disease trends in a larger area. A sentinel approach is useful when high-quality data is needed that cannot be obtained through passive surveillance [89] and the workload and/or costs of national surveillance is too high. The methodology of an epidemiological surveillance system is adapted to its aim; control of an infectious disease with a high disease burden requires comprehensive national surveillance, whereas elimination of a rare infectious disease requires more detailed information [89]. A surveillance system can also be applied at a regional or local level, e.g. within a single hospital, to guide the local infection control measures.

INTRODUCTION OF NATIONAL SURVEILLANCE OF CDI

Historically, the use of epidemiological surveillance systems for CDI has been triggered by the occurrence of outbreaks. One of the first publications on surveillance of CDI describes the implementation of active hospital-wide surveillance after the recognition of a CDI cluster in Belgium in 1988 [90]. Interestingly, the authors note that surveillance outcomes were initially biased due to unawareness and underdiagnosis of CDI. After addressing these issues, surveillance and molecular typing led to improved prevention of CDI. In the Netherlands, a first laboratory-based surveillance study was conducted in 2005 [91]. This study coincided with the occurrence of PCR ribotype 027 CDI outbreaks in the Netherlands [92]. In 2006-2009, Dutch national surveillance was limited to severe cases of CDI and outbreaks and a three-year surveillance study [93]. In 2009, continued Sentinel Surveillance for CDI was implemented in a subset of hospitals in the Netherlands (Figure 2). Aims are to:

- obtain continuous incidence rates of CDI in hospitals in the Netherlands
- identify and characterize new circulating PCR ribotypes
- correlate new circulating PCR ribotypes with changes of epidemiology, and clinical syndromes of CDI

The Dutch Sentinel Surveillance for CDI is a disease-specific, healthcare-provider based surveillance system that targets acute care hospitals only. Inclusion of patients is based on the presence of clinical symptoms of CDI and laboratory confirmation. The surveillance system is exceptional in Europe for its unselective PCR ribotyping of all submitted *C. difficile* strains. Hospital participation in Sentinel Surveillance for CDI is voluntary. Outcomes of Sentinel Surveillance for CDI are used to prioritise and monitor national control of CDI, especially transmission and outbreaks of ribotype 027 and other *C. difficile* strains with an increased ecological fitness. Hospital get direct notification of molecular typing results in case of ribotype 027 and/or outbreaks and can receive additional support for local infection control measures. Healthcare facilities not participating in Sentinel Surveillance for CDI are able to submit samples for (free of charge) PCR ribotyping in case of severe CDI or suspected outbreaks.

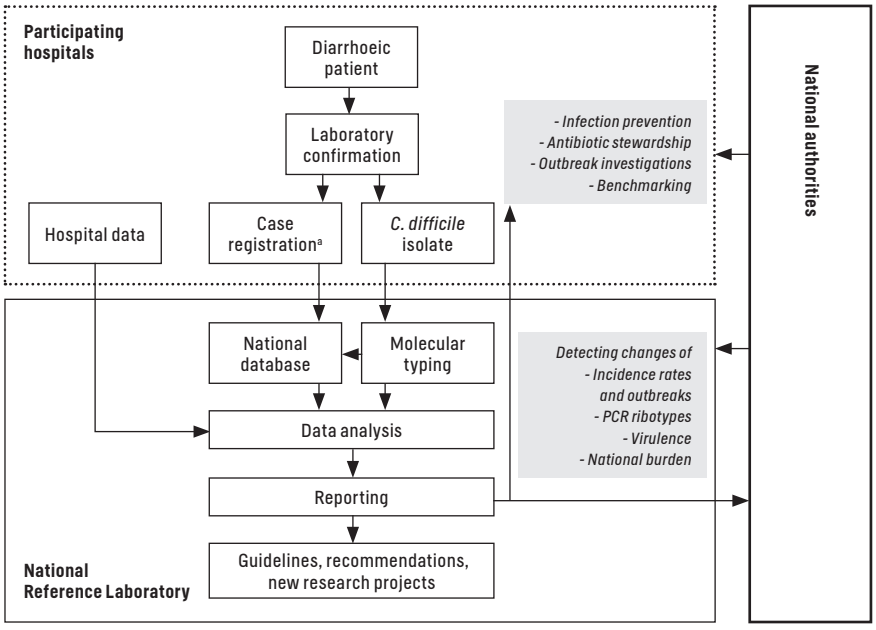

Figure 2 Flow chart of Sentinel Surveillance for CDI in the Netherlands. The National Reference Laboratory for *C. difficile* is a collaboration of the Department of Medical Microbiology of the Leiden University Medical Centre in Leiden and the National Institute for Public Health and the Environment (RIVM) in Bilthoven. [a]The Orisis registration system of the RIVM is used for case registration with clinical and epidemiological data, and linkage of molecular typing results.

SURVEILLANCE OF CDI IN EUROPE

In Europe, national surveillance activities of European countries were supported by ECDC after recognition of the emergence of ribotype 027 in Europe in 2006, gradually moving towards standardised epidemiological surveillance and molecular typing systems [4]. In the same year, ECDC and the ESCMID study group for *C. difficile* publised 'interim' surveillance definitions for CDI [3]. European countries were advised to adapt their national surveillance system to their local situation, selecting for laboratory-based surveillance or healthcare provider-based surveillance in specific, targeted populations. In 2005, a first pilot of multicountry two-month survey in Europe was conducted [94]. This study underlined the need for implementation of a standardised case-definition and harmonisation of laboratory diagnostics for benchmarking. The need for routine surveillance was reinforced in the ESCMID guideline to control transmission of CDI in 2008, also in the absence of outbreaks [95]. In that year, a first periodic European surveillance for CDI illustrated the added value of multicountry surveillance in Europe [64]. However, the continued heterogeneity of diagnostic, molecular typing and surveillance methodologies hampered implementation of European-wide surveillance.

By way of comparison, The Centers for Disease Control and Prevention (CDC) integrated CDI surveillance as separate component in the Healthcare-Associated Infections Community Interface (HAIC) of the Emerging Infections Program (EIP) in the United in 2009 [96]. Before that time, surveillance was implemented in the National Nosocomial Infection Surveillance System initiated in 1970 [97]. The CDI surveillance component of EIP is population-based and aims to estimate the burden of health-care associated CDI and in the community, describe other epidemiological aspects of CDI and characterize *C. difficile* strains [96].
In 2011, 14 of the 31 European countries (45%) had adopted CDI surveillance and methodologies were heterogeneous. Continued integration of microbiological data was limited [98]. A multistate CDI surveillance system, such as implemented in the United States[99], was considered the only viable option to monitor and control CDI in Europe. Objectives of such a European surveillance system of CDI were agreed to [100]:

- estimate the incidence of CDI in European acute care hospitals
- assess the burden of CDI in European acute care hospitals
- provide participating hospitals with a standardised tool to measure and compare their own incidence rates with those observed in other participating hospitals
- assess adverse outcomes of CDI including death
- describe the epidemiology of *C. difficile* at the local, national and European level, in terms of factors such as antibiotic susceptibility, PCR ribotype, presence of toxin A (TcdA), toxin B (TcdB) and binary toxin, morbidity and mortality of infection, and the detection of new/emerging types

The 'European CDI Surveillance Network' (ECDIS-Net) aimed to optimise and test the feasibility of a surveillance protocol for European Surveillance of CDI in 2010-2014.

USE OF MOLECULAR TYPING FOR SURVEILLANCE

Molecular typing methods aim to identify relatedness of pathogens and are used to test epidemiological hypotheses on transmission events [101]. Hence, molecular typing is vital for outbreak investigations [102]. Molecular typing also supports monitoring of 'outbreak-associated' or more virulent genotypes in an endemic setting. Several typing methodologies for *C. difficile* were developed and implemented all over the world, targeting different parts of the *C. difficile* genome [103 – 105]. PCR ribotyping is the traditional typing method in Europe. It targets the intermediary region of the 16S and 23S rRNA genes with a variable length, present in multiple copies of the ribosomal operon in the genome [106, 107]. Identical PCR ribotypes have an equivalent banding pattern after visualising the DNA fragments [106]. Currently, 219 PCR ribotypes can be discriminated by the national reference laboratory for *C. difficile* in the Netherlands [108]. Capillary gel-based electrophoresis ribotyping has an improved performance compared to conventional (agarose gel-based) PCR ribotyping for inter-laboratory standardization [109]. Moreover, the electronic portability of capillary ribotyping results is considered to dissolve the current problem of limited reference/central databases and contribute to more rapid detection of internationally emerging PCR ribotypes [109]. *C. difficile* transmission events are hallmarked by identical PCR ribotypes, but far more discriminatory typing methods are required to confirm transmission events and to study outbreaks [103, 105]. One of these methods is Multiple-Locus Variable-Number Tandem-Repeat Analysis (MLVA), targeting seven regions with short tandem repeats spread in the *C. difficile* genome [110]. The total number of differences in repeat copy number at each locus (summed tandem-repeat difference; STRD) describes the genetic relatedness of the studies isolates [111]. Whole genome sequencing –of which the application for *C. difficile* was firstly reported in 2010 [112]– has the capability to distinct strains at a single nucleotide level; single nucleotide variants (SNV) [103]. The application of whole-genome sequencing led to important insights in the evolution and (drivers of) transmission of CDI [2, 50, 112].

The burden of CDI in hospitals

After the discovery of *C. difficile* as a pathogen in 1978, CDI was soon recognised as the foremost cause of antibiotic-associated diarrhoea in hospitals, causing 15-25% of all cases [102]. The regional incidence rate of CDI ranged from 1.1-7.9 cases per 10,000 patient-days in the United States according to data from the National Nosocomial Infections Surveillance System in 1978-2001 [97]. A gradual increase of the incidence of CDI was noted for IC units and smaller hospitals. In Canada, the incidence rate of CDI was estimated at 6 cases per 10,000 patient-days in 1997 (corresponding to a prevalence of 13% among all diarrhoeic inpatients) by surveillance part of the Canadian Nosocomial Infection Surveillance Program [113]. In 2002, the incidence rate of CDI increased dramatically in the region of Quebec in Canada to 15 cases per 10,000 patient-days [114, 115]. Almost a quart (23%) of the affected patients died within 30-days compared to 7% of matched controls [115]. Continuing signs of increased severity of CDI and the occurrence of outbreaks led to the recognition of the emergence of an earlier uncommon ribotype 027/NAP 1 strain in Canada and the United States in 2005 [116, 117].

In the Netherlands, the endemic incidence of CDI remained unreported until 2005 but sporadic outbreaks were noticed [118 – 120]. In 2005, an excessive number of CDI outbreaks occurred with a high impact on the local incidence rates and mortality [91, 121 – 125]. Also other European countries, i.e. the United Kingdom, Belgium and France, were affected by CDI outbreaks [94]. These outbreaks were related to the global spread of the ribotype 027/NAP1 strain from Canada and the United States towards Europe [2]. Transmission of ribotype 027 had been reported by 11 and 16 European countries in 2007 and 2008, respectively [126]. Introduction of ribotype 027 could be linked to international travels in some countries. The exceptional virulence of ribotype 027 was attributed to increased toxin production (associated to its *tcdC* gene mutation) and altered antibiotic resistance [126]. Retrospective investigation using whole-genome sequencing demonstrated that fluoroquinolone resistance acquired by two separate ribotype 027/NAP1 lineages contributed to its emergence and undermined the role of the *tcdC* gene mutation [2].

The Netherlands was the first European country that reported a decrease of ribotype 027 in 2006 by national surveillance data [93]. The incidence rate of CDI in hospitals in the Netherlands stabilised at 3 cases per 10,000 patient-days and ribotype 027 caused not more than 3% of all CDI (virtually all relating to healthcare). However, transmission of ribotype 078 caused concern due it high abundance, especially compared to other countries [93]. A European surveillance estimated the incidence of healthcare-associated CDI at 4 cases per 10,000 patient-days in 2008. Ribotype 027 was isolated from 5% of CDI, and less prevalent than e.g. ribotype 078 [64] The United Kingdom had a remarkable high prevalence of ribotype 027, but succeeded to reduce the national incidence rate of CDI and prevalence of ribotype 027 by antibiotic stewardship (e.g. restricting fluoroquinolone prescribing) in additional to other infection prevention control measures in 2007-2013 [127]. ECDC estimated the overall number of healthcare-acquired CDI at

123,997 (95% CI: 107,697-441,969) in 2011-2012 [128]. Yet, thereafter ribotype 027 emerged in Eastern-Europe [129].

In the United States however, 31% of healthcare-associated CDI and 19% of community-associated CDI was caused by ribotype 027/NAP1 in 2011 [99]. It was estimated that approximately 453,000 patients were affected by CDI in 2011 (95% confidence interval: 397,100 -508,500) of which 29,000 succumbed (Figure 3). CDC denoted CDI as one of the three urgent 'antibiotic resistance threats' in the United States in 2013 [130].

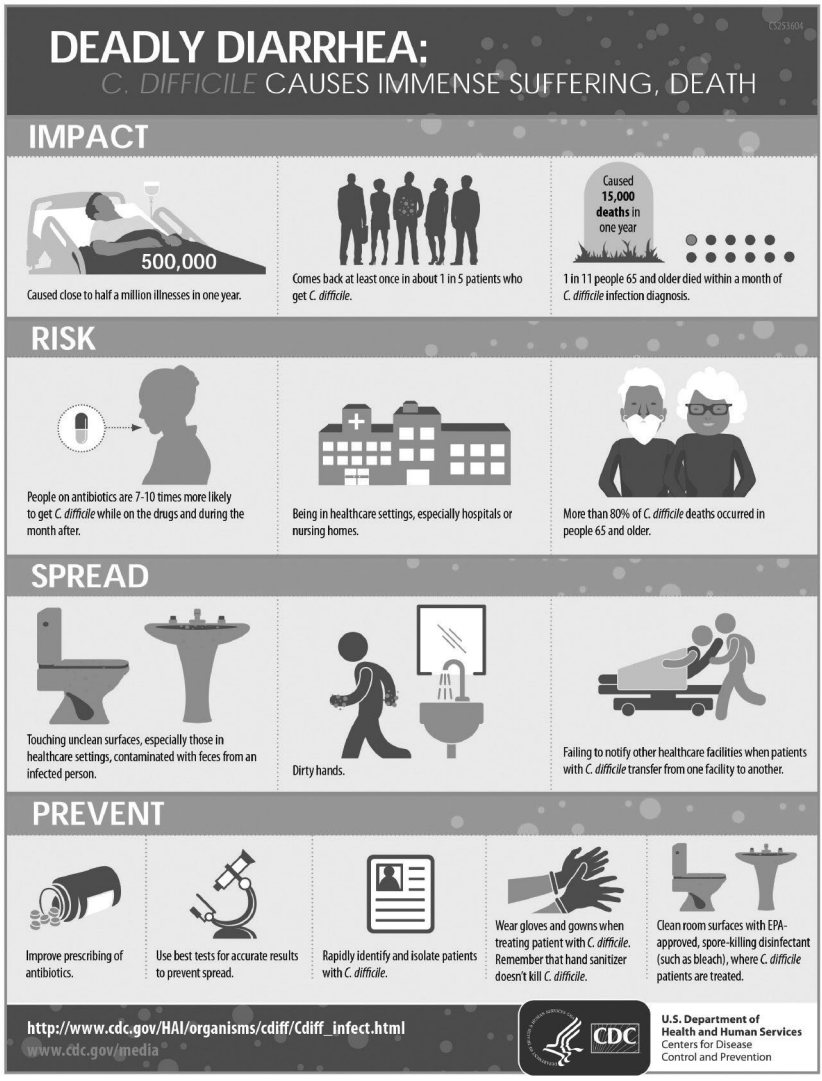

Figure 3 Infographic of the Centers for Disease Control and Prevention (CDC) on prevention of CDI.

CDI beyond hospitals

As hospitals, long-term care facilities (LTCF) host a population at high risk for developing CDI, due to frequent antibiotic consumption, advanced age, comorbidities and suboptimal infection prevention. LTCF residents can facilitate *C. difficile* transmission between hospitals and LTCF [111]. In the United States, the number of CDI cases in nursing homes in 2012 was estimated at 112,800 (95% confidence interval: 93,400-131,800), circa one quarter of all CDI cases [131]. These numbers may be biased due to the fact that *C. difficile* laboratory testing in nursing homes is not always part of daily routine. A large proportion of the patients (76%) had been hospitalised ≤12 weeks before CDI and the very old (>85 yrs) were at particular risk [131]. In Europe, the burden of healthcare-associated infections in LTCF is monitored by repeated point-prevalence surveys. CDI was not one of most common HAI or isolated microorganisms. The CDI-specific burden was not reported but approximated 37,900 (0.9% of all HAI) on the basis of available data [132]. There are no national estimates of CDI in LTCF in the Netherlands, but outbreaks have been reported [3].

In the general population, the incidence of CDI in the community was estimated at 0.67 cases per 10,000 person years (95% CI 0.58–0.78) in the Netherlands in 2010-2012, comparable to *Salmonella* spp. This corresponded to a prevalence of 1.5% amongst community residents that visit their general practitioner with diarrhoea and submit a stool sample for laboratory testing [59, 60]. In another Dutch study, 4.2% of community residents with gastro-intestinal complaints were positive for toxigenic *C. difficile* using PCR, higher than *Salmonella* spp [133]. In Denmark, the community incidence of CDI was 2.3 cases per 10,000 person years [58]. In Minnesota, United States, the incidence of community-acquired CDI was estimated at 0.96 cases per 10,000 person years in 1991-2005 in a population-based study [134]. While recent literature underlines the increasing burden of CDI in the community, the substantial community burden of CDI has been confirmed decades ago –prior to the emergence of ribotype 027/NAP1–if tested for [135]. Yet, the minority of the diarrhoeic community patients were tested for CDI at that time [135, 136].

Community patients with CDI have a rather different risk profile compared to hospital patients [134, 137]. Antibiotic use is the foremost risk-factor for CDI as in hospitalised patients, but absent in a considerable proportion (30-60%) of the community patients [59, 60, 137, 138]. It was suggested that other disrupting factors of the microbiome cause CDI in this subpopulation of non-exposed patients (e.g. proton-pump inhibiters) [139]. As mentioned before, *C. difficile* reservoirs include asymptomatic infant and adult carriers, and animals (e.g. pets). Food, water and other environmental contamination can be considered as 'sinks' rather than reservoirs, but may be part of CDI transmission paths. In previous years, molecular studies aimed to trace CDI sources. One of these studies showed that 13% of CDI in adults were genetically related to infants strains according to whole-genome sequencing [140]. Advanced age, breastfeeding and exposure to pet dogs were

found to be risk factors for *C. difficile* in children in the same study. Animal contact has been recognised to increase the risk of CDI in children <2 yrs old by others [138]. Phylogenetic results of other whole-genome sequence studies suggest that numerous long-range transmission events occur between pet dogs and humans [141], as well as pigs and humans [142, 143]. Transmission paths have not been elucidated. In the Netherlands, piglets are frequently colonised by *C. difficile* PCR ribotype 078 [144] and contamination of the farm environment has been demonstrated [145]. Persons with daily contact with pigs had a 1:4 risk to be positive for *C. difficile* in a small study, virtually all ribotype 078 [146]. In Central North Carolina, one of the largest pig producing states in the United States, environmental exposure to livestock farms increases the risk for community-acquired CDI [147]. Moreover, CDI complies with some criteria of foodborne disease. Yet, the attribution of food to CDI transmission is considered low, as illustrated for hospitalised patients [148]. *C. difficile* cannot germinate and grow in food, and outbreaks of CDI were never found to be food-related [37, 149]. Yet, regular consumption of beef was an imported risk factor (Odds Ratio 5.5) of community-acquired CDI for adults in Denmark in contrast to other food products [138]. Overall, a complex interplay of animal and human reservoir populations and environmental sources ('sinks') need to be considered for CDI transmission beyond hospitals.

Outline of the thesis

This thesis aims to describe contemporary changes of the epidemiology of CDI in the Netherlands and Europe and the subsequent introduction of a new standardised epidemiological surveillance system for CDI in Europe. Findings will be used to guide future directions of epidemiological surveillance systems for CDI –in particular for Europe and the Netherlands. This will improve estimations of the infection burden and helps to understand *C. difficile* sources and transmission routes that are needed for appropriate infection prevention control interventions.

The heterogeneity of existing epidemiological surveillance systems for CDI in Europe hampered a valid estimation of CDI burden and illustrated the need for a standardised European-wide surveillance system for CDI. However, suboptimal laboratory diagnostic capacity was considered as the foremost barrier for implementing European-wide surveillance for CDI. Besides, application of various non-standardised molecular typing methodologies prevented their use for monitoring transmission and control. In 2010, ECDC supported a 4-year project named 'the European CDI Surveillance Network' (ECDIS-Net) to enhance CDI surveillance and laboratory capacity to test for CDI. This thesis incorporates two studies that were conducted within the framework of ECDIS-Net. **CHAPTER 2** evaluates changes in local laboratory diagnostic and national typing capacity for CDI through cross-sectional surveys amongst ECDIS-Net participants in 33 European countries in 2011 and 2014. **CHAPTER 3** explores the feasibility of implementing a standardised European surveillance protocol for CDI through a three-month pilot in 14 countries in 2013. This study also illustrates the added value of collecting detailed epidemiological and microbiological data on CDI at European level. ECDIS-Net activities resulted in initiation of European Surveillance of CDI in EU/EEA countries by ECDC in 2016.

The Netherlands has a national reference laboratory in place to support epidemiological surveillance and molecular typing of CDI since the recognition of ribotype 027 outbreaks in 2005. A national Sentinel Surveillance for CDI was implemented for ongoing monitoring of the incidence of CDI and detection of new outbreaks in 2009. According to this surveillance, the incidence of CDI stabilised at 3 cases per 10,000 patient-days. Yet, the burden of CDI in children was never examined in detail but has been reported to increase in other countries. **CHAPTER 4** investigates changes of the number of reported CDI amongst children in the Netherlands. Additionally, the clinical and microbiological characteristics of CDI in hospitalised children are compared to adults to determine if additional strategies to prevent, diagnose, and treat CDI in children are needed. Overall ribotype 027 caused not more than 3% of all CDI, but the high incidence of ribotype 078 caused concern because of its potential relation to pig-farming. **CHAPTER 5** assesses the association between hospital incidence rates or ribotype 078 and provincial pig-farming. This study also incorporates the use of spatial

scan statistics to search for clustering of community-CDI that could indicate sources of CDI beyond hospitals. **CHAPTER 6** extends the use of spatial scan statistics in a community-based case-control study of CDI, with detailed data on environmental exposure of community-acquired CDI patients. These data are used to test livestock exposure as a risk factor for community-acquired CDI.

CHAPTER 7 elaborates on how advanced insights in the epidemiology, sources and transmission of CDI challenge present surveillance systems and synthesises future directions for improved surveillance systems and control of CDI.

References

1. Bartlett JG, Moon N, Chang TW, Taylor N, Onderdonk AB. Role of *Clostridium difficile* in antibiotic-associated pseudomembranous colitis. Gastroenterology. 1978;75(5):778-82.

2. He M, Miyajima F, Roberts P, Ellison L, Pickard DJ, Martin MJ, et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. Nat Genet. 2013;45(1):109-13.

3. Kuijper EJ, Coignard B, Tüll P, ESCMID Study Group for *Clostridium difficile*, EU Member States, European Centre for Disease Prevention and Control. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect. 2006;12 Suppl 6:2-18.

4. Suetens C. *Clostridium difficile*: summary of actions in the European Union. Euro Surveill. 2008;13(31).

5. Hall IC, O'Toole E. Intestinal flora in new-born infants: with a description of a new pathogenic anaerobe, *Bacillus difficilis*. Am J Child Dis. 1935;49:390–402.

6. Furuya-Kanamori L, Marquess J, Yakob L, Riley TV, Paterson DL, Foster NF, et al. Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications. BMC Infect Dis. 2015;15:516.

7. Lees EA, Miyajima F, Pirmohamed M, Carrol ED. The role of *Clostridium difficile* in the paediatric and neonatal gut - a narrative review. Eur J Clin Microbiol Infect Dis. 2016;35(7):1047-57.

8. Fleming A. Twentieth-century changes in the treatment of septic infections. N Engl J Med. 1953; 248(25):1037-45.

9. Bartlett JG. Historical perspectives on studies of *Clostridium difficile* and *C. difficile* infection. Clin Infect Dis. 2008;46:S4-S11.

10. Smith LD, King EO. Occurrence of *Clostridium difficile* in infections of man. J Bacteriol. 1962;84:65-7.

11. McFarland LV, Stamm WE. Review of *Clostridium difficile*-associated diseases. Am J Infect Control. 1986;14(3):99-109.

12. Tedesco FJ, Stanley RJ, Alpers DH. Diagnostic features of clindamycin-associated pseudomembranous colitis. N Engl J Med. 1974;290(15):841-3.

13. Tedesco FJ. Clindamycin-associated colitis. Review of the clinical spectrum of 47 cases. Am J Dig Dis. 1976;21(1):26-32.

14. Popoff MR. From saprophytic to toxigenic clostridia, a complex evolution based on multiple diverse genetic transfers and/or rearrangements. Res Microbiol. 2015;166(4):221-4.

15. Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prevot 1938. Anaerobe. 2016;40:95-9.

16. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. Gastroenterology. 2014;146(6):1547-53.

17. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. Am J Physiol Gastrointest Liver Physiol. 2014;306(4):G310-9.

18. Hensgens MP, Goorhuis A, Dekkers OM, Kuijper EJ. Time interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics. J Antimicrob Chemother. 2012;67(3):742-8.

19. Dial S, Kezouh A, Dascal A, Barkun A, Suissa S. Patterns of antibiotic use and risk of hospital admission because of *Clostridium difficile* infection. CMAJ. 2008;179(8):767-72.

20. Stevens V, Dumyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of *Clostridium difficile* infection. Clin Infect Dis. 2011;53(1):42-8.

21. Slimings C, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile* infection: update of systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(4):881-91.

22. Deshpande A, Pasupuleti V, Thota P, Pant C, Rolston DD, Sferra TJ, et al. Community-associated *Clostridium difficile* infection and antibiotics: a meta-analysis. J Antimicrob Chemother. 2013;68(9):1951-61.

23. Imhann F, Vich Vila A, Bonder MJ, Lopez Manosalva AG, Koonen DP, Fu J, et al. The influence of proton pump inhibitors and other commonly used medication on the gut microbiota. Gut Microbes. 2017:1-8.

24. Elliott B, Androga GO, Knight DR, Riley TV. *Clostridium difficile* infection: Evolution, phylogeny and molecular epidemiology. Infect Genet Evol. 2016; 49:1-11.

25. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nat Rev Dis Primers. 2016;2:16020.

26. Dingle KE, Elliott B, Robinson E, Griffiths D, Eyre DW, Stoesser N, et al. Evolutionary history of the *Clostridium difficile* pathogenicity locus. Genome Biol Evol. 2014;6(1):36-52.

27. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP. The role of toxin A and toxin B in *Clostridium difficile* infection. Nature. 2010;467 (7316):711-3.

28. Chumbler NM, Farrow MA, Lapierre LA, Franklin JL, Lacy DB. *Clostridium difficile* Toxins TcdA and TcdB Cause Colonic Tissue Damage by Distinct Mechanisms. Infect Immun. 2016;84(10):2871-7.

29. Gerding DN, Johnson S, Rupnik M, Aktories K. *Clostridium difficile* binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut Microbes. 2014;5(1):15-27.

30. Schwan C, Kruppke AS, Noelke T, Schumacher L, Koch-Nolte F, Kudryashev M, et al. *Clostridium difficile* toxin CDT hijacks microtubule organization and reroutes vesicle traffic to increase pathogen adherence. Proc Natl Acad Sci U S A. 2014;111(6):2313-8.

31. Hensgens MP, Kuijper EJ. *Clostridium difficile* infection caused by binary toxin-positive strains. Emerg Infect Dis. 2013;19(9):1539-40.

32. Awad MM, Johanesen PA, Carter GP, Rose E, Lyras D. *Clostridium difficile* virulence factors: Insights into an anaerobic spore-forming pathogen. Gut Microbes. 2014;5(5):579-93.

33. Corver J, Cordo V, van Leeuwen HC, Klychnikov OI, Hensbergen PJ. Covalent attachment and Pro-Pro endopeptidase (PPEP-1)-mediated release of *Clostridium difficile* cell surface proteins involved in adhesion. Mol Microbiol. 2017;105(5):663-73.

34. Robinson CD, Auchtung JM, Collins J, Britton RA. Epidemic *Clostridium difficile* strains demonstrate increased competitive fitness compared to nonepidemic isolates. Infect Immun. 2014;82(7):2815-25.

35. Paredes-Sabja D, Shen A, Sorg JA. *Clostridium difficile* spore biology: sporulation, germination, and spore structural proteins. Trends Microbiol. 2014; 22(7):406-16.

36. Gil F, Lagos-Moraga S, Calderon-Romero P, Pizarro-Guajardo M, Paredes-Sabja D. Updates on *Clostridium difficile* spore biology. Anaerobe. 2017.

37. Hensgens MP, Keessen EC, Squire MM, Riley TV, Koene MG, de Boer E, et al. *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012;18(7):635-45.

38. Donskey CJ, Kundrapu S, Deshpande A. Colonization versus carriage of *Clostridium difficile*. Infect Dis Clin North Am. 2015;29(1):13-28.

39. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RLP, Donskey CJ. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic *Clostridium difficile* strains among long-term care facility residents. Clin Infect Dis. 2007; 45(8):992-8.

40. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. Lancet. 1998;351(9103):633-6.

41. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N Engl J Med. 2000;342(6):390-7.

42. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, et al. Host and pathogen factors for *Clostridium difficile* infection and colonization. N Engl J Med. 2011;365(18):1693-703.

43. Forster AJ, Daneman N, van Walraven C. Influence of antibiotics and case exposure on hospital-acquired *Clostridium difficile* infection independent of illness severity. J Hosp Infect. 2017;95(4):400-9.

44. Dubberke ER, Reske KA, Yan Y, Olsen MA, McDonald LC, Fraser VJ. *Clostridium difficile*-associated disease in a setting of endemicity: identification of novel risk factors. Clin Infect Dis. 2007;45(12): 1543-9.

45. Louh IK, Greendyke WG, Hermann EA, Davidson KW, Falzon L, Vawdrey DK, et al. *Clostridium Difficile* Infection in Acute Care Hospitals: Systematic Review and Best Practices for Prevention. Infect Control Hosp Epidemiol. 2017;38(4):476-82.

46. Feazel LM, Malhotra A, Perencevich EN, Kaboli P, Diekema DJ, Schweizer ML. Effect of antibiotic stewardship programmes on *Clostridium difficile* incidence: a systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(7):1748-54.

47. Davey P, Marwick CA, Scott CL, Charani E, McNeil K, Brown E, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database Syst Rev. 2017;2:CD003543.

48. Khanafer N, Voirin N, Barbut F, Kuijper E, Vanhems P. Hospital management of *Clostridium difficile* infection: a review of the literature. J Hosp Infect. 2015.

49. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect Control Hosp Epidemiol. 2010;31(5):431-55.

50. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, et al. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med. 2013;369(13):1195-205.

51. Kumar N, Miyajima F, He M, Roberts P, Swale A, Ellison L, et al. Genome-Based Infection Tracking Reveals Dynamics of *Clostridium difficile* Transmission and Disease Recurrence. Clin Infect Dis. 2016;62 (6):746-52.

52. Eyre DW, Fawley WN, Rajgopal A, Settle C, Mortimer K, Goldenberg SD, et al. Comparison of Control of *Clostridium difficile* Infection in Six English Hospitals Using Whole-Genome Sequencing. Clin Infect Dis. 2017;65(3):433-41.

53. Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. Identifying reservoirs of infection: a conceptual and practical challenge. Emerg Infect Dis. 2002;8(12) :1468-73.

54. Gupta A, Khanna S. Community-acquired *Clostridium difficile* infection: an increasing public health threat. Infect Drug Resist. 2014;7:63-72.

55. Alam MJ, Walk ST, Endres BT, Basseres E, Khaleduzzaman M, Amadio J, et al. Community Environmental Contamination of Toxigenic *Clostridium difficile*. Open forum infectious diseases. 2017;4(1): ofx018.

56. Dantes R, Mu Y, Hicks LA, Cohen J, Bamberg W, Beldavs ZG, et al. Association Between Outpatient Antibiotic Prescribing Practices and Community-Associated *Clostridium difficile* Infection. Open forum infectious diseases. 2015;2(3).

57. Farooq PD, Urrunaga NH, Tang DM, von Rosenvinge EC. Pseudomembranous colitis. Dis Mon. 2015;61(5):181-206.

58. Søes LM, Holt HM, Bottiger B, Nielsen HV, Torpdahl M, Nielsen EM, et al. The incidence and clinical symptomatology of *Clostridium difficile* infections in a community setting in a cohort of Danish patients attending general practice. Eur J Clin Microbiol Infect Dis. 2014;33(6):957-67.

59. Hensgens MP, Dekkers OM, Demeulemeester A, Buiting AG, Bloembergen P, van Benthem BH, et al. Diarrhoea in general practice: when should a *Clostridium difficile* infection be considered? Results of a nested case-control study. Clin Microbiol Infect. 2014.

60. Bauer MP, Veenendaal D, Verhoef L, Bloembergen P, van Dissel JT, Kuijper EJ. Clinical and microbiological characteristics of community-onset *Clostridium difficile* infection in The Netherlands. Clin Microbiol Infect. 2009;15(12):1087-92.

61. Bauer MP, Goorhuis A, Koster T, Numan-Ruberg SC, Hagen EC, Debast SB, et al. Community-onset *Clostridium difficile*-associated diarrhoea not associated with antibiotic usage--two case reports with review of the changing epidemiology of *Clostridium difficile*-associated diarrhoea. Neth J Med. 2008;66 (5):207-11.

62. Bagdasarian N, Rao K, Malani PN. Diagnosis and treatment of *Clostridium difficile* in adults: a systematic review. JAMA. 2015;313(4):398-408.

63. Hensgens MP, Goorhuis A, van Kinschot CM, Crobach MJ, Harmanus C, Kuijper EJ. *Clostridium difficile* infection in an endemic setting in the Netherlands. Eur J Clin Microbiol Infect Dis. 2011;30 (4):587-93.

64. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377(9759):63-73.

65. Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper EJ. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study. Clin Infect Dis. 2013;56(8):1108-16.

66. McFarland LV, Ozen M, Dinleyici EC, Goh S. Comparison of pediatric and adult antibiotic-associated diarrhea and *Clostridium difficile* infections. World J Gastroenterol. 2016;22(11):3078-104.

67. Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). Clin Microbiol Infect. 2009;15(12): 1053-66.

68. Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2016;22 Suppl 4:S63-81.

69. Debast SB, Bauer MP, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014;20 Suppl 2:1-26.

70. SWAB. Optimaliseren van het antibioticabeleid in Nederland XVIII. SWAB richtlijn antimicrobiële therapie voor acute infectieuze diarree. Nijmegen 2014.

71. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. Am J Gastroenterol. 2013;108(4):478-98; quiz 99.

72. Edwards DI. Nitroimidazole drugs--action and resistance mechanisms. I. Mechanisms of action. J Antimicrob Chemother. 1993;31(1):9-20.

73. Barna JC, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Annu Rev Microbiol. 1984;38:339-57.

74. Nelson RL, Suda KJ, Evans CT. Antibiotic treatment for *Clostridium difficile*-associated diarrhoea in adults. Cochrane Database Syst Rev. 2017;3:CD00 4610.

75. Lewis BB, Buffie CG, Carter RA, Leiner I, Toussaint NC, Miller LC, et al. Loss of Microbiota-Mediated Colonization Resistance to *Clostridium difficile* Infection With Oral Vancomycin Compared With Metronidazole. J Infect Dis. 2015;212(10):1656-65.

76. Freeman J, Vernon J, Morris K, Nicholson S, Todhunter S, Longshaw C, et al. Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes. Clin Microbiol Infect. 2015;21(3):248-.

77. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. N Engl J Med. 2011; 364(5):422-31.

78. Vardakas KZ, Polyzos KA, Patouni K, Rafailidis PI, Samonis G, Falagas ME. Treatment failure and recurrence of *Clostridium difficile* infection following treatment with vancomycin or metronidazole: a systematic review of the evidence. Int J Antimicrob Agents. 2012;40(1):1-8.

79. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013;368(5):407-15.

80. Terveer EM, van Beurden YH, Goorhuis A, Seegers J, Bauer MP, van Nood E, et al. How to: Establish and run a stool bank. Clin Microbiol Infect. 2017;23(12):924-30.

81. Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for Prevention of Recurrent *Clostridium difficile* Infection. N Engl J Med. 2017;376(4):305-17.

82. Gerding DN, Meyer T, Lee C, Cohen SH, Murthy UK, Poirier A, et al. Administration of spores of nontoxigenic *Clostridium difficile* strain M3 for prevention of recurrent *C. difficile* infection: a randomized clinical trial. JAMA. 2015;313(17):1719-27.

83. Fehér C, Soriano A, Mensa J. A Review of Experimental and Off-Label Therapies for *Clostridium difficile* Infection. Infect Dis Ther. 2016.

84. Khanna S, Pardi DS, Kelly CR, Kraft CS, Dhere T, Henn MR, et al. A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent *Clostridium difficile* Infection. J Infect Dis. 2016;214(2):173-81.

85. Langmuir AD. The surveillance of communicable diseases of national importance. N Engl J Med. 1963;268:182-92.

86. Thacker SB, Parrish RG, Trowbridge FL. A method for evaluating systems of epidemiological surveillance. World Health Stat Q. 1988;41(1):11-8.

87. Abat C, Chaudet H, Rolain JM, Colson P, Raoult D. Traditional and syndromic surveillance of infectious diseases and pathogens. Int J Infect Dis. 2016;48:22-8.

88. Sickbert-Bennett EE, Weber DJ, Poole C, MacDonald PD, Maillard JM. Completeness of communicable disease reporting, North Carolina, USA, 1995-1997 and 2000-2006. Emerg Infect Dis. 2011; 17(1):23-9.

89. WHO. Types of surveillance. Available from: http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/en/.

90. Struelens MJ, Maas A, Nonhoff C, Deplano A, Rost F, Serruys E, et al. Control of nosocomial transmission of *Clostridium difficile* based on sporadic case surveillance. Am J Med. 1991;91(3B):138S-44S.

91. Paltansing S, van den Berg RJ, Guseinova RA, Visser CE, van der Vorm ER, Kuijper EJ. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. Clin Microbiol Infect. 2007;13(11):1058-64.

92. Notermans DW, van der Kooi TI, Goorhuis A, Debast SB, van Benthem BH, Kuijper EJ. Epidemiology of *Clostridium difficile* PCR ribotype 027 in the Netherlands 2005-present and the emergence of other subtypes. Ned Tijdschr Geneeskd. 2008;152 (35):1937-40.

93. Hensgens MP, Goorhuis A, Notermans DW, van Benthem BH, Kuijper EJ. Decrease of hypervirulent *Clostridium difficile* PCR ribotype 027 in the Netherlands. Euro Surveill. 2009;14(45).

94. Barbut F, Mastrantonio P, Delmee M, Brazier J, Kuijper E, Poxton I, et al. Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterisation of the isolates. Clin Microbiol Infect. 2007;13(11):1048-57.

95. Vonberg RP, Kuijper EJ, Wilcox MH, Barbut F, Tüll P, Gastmeier P, et al. Infection control measures to limit the spread of *Clostridium difficile*. Clin Microbiol Infect. 2008;14 Suppl 5:2-20.

96. Magill SS, Dumyati G, Ray SM, Fridkin SK. Evaluating Epidemiology and Improving Surveillance of Infections Associated with Health Care, United States. Emerg Infect Dis. 2015;21(9):1537-42.

97. Archibald LK, Banerjee SN, Jarvis WR. Secular trends in hospital-acquired *Clostridium difficile* disease in the United States, 1987-2001. J Infect Dis. 2004;189(9):1585-9.

98. Kola A, Weitzel-Kage D, van Benthem BH, Coignard B, Lyytikainen O, Struwe J, et al. Surveillance of *Clostridium difficile* infections: literature review and survey of surveillance systems. 2014.

99. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med. 2015;372(9):825-34.

100. European Centre for Disease Prevention and Control. European Surveillance of *Clostridium difficile* infections. Surveillance protocol version 2.2. Stockholm: ECDC; 2015.

101. Field N, Cohen T, Struelens MJ, Palm D, Cookson B, Glynn JR, et al. Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID): an extension of the STROBE statement. Lancet Infect Dis. 2014;14(4):341-52.

102. Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J, Jr. *Clostridium difficile*-associated diarrhea and colitis. Infect Control Hosp Epidemiol. 1995;16(8):459-77.

103. Knetsch CW, Lawley TD, Hensgens MP, Corver J, Wilcox MW, Kuijper EJ. Current application and future perspectives of molecular typing methods to study *Clostridium difficile* infections. Euro Surveill. 2013;18(4):20381.

104. Huber CA, Foster NF, Riley TV, Paterson DL. Challenges for standardization of *Clostridium difficile* typing methods. J Clin Microbiol. 2013;51(9):2810-4.

105. Killgore G, Thompson A, Johnson S, Brazier J, Kuijper E, Pepin J, et al. Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile*: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. Journal of clinical microbiology. 2008;46(2):431-7.

106. Bidet P, Lalande V, Salauze B, Burghoffer B, Avesani V, Delmée M, et al. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing *Clostridium difficile*. J Clin Microbiol. 2000;38(7):2484-7.

107. Gürtler V. Typing of *Clostridium difficile* strains by PCR-amplification of variable length 16S-23S rDNA spacer regions. J Gen Microbiol. 1993;139 (12):3089-97.

108. Crobach MJT, van Dorp SM, Harmanus C, Sanders IMJG, Kuijper EJ, Notermans DW, et al. Eleventh Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance May 2016 - May 2017. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2017.

109. Fawley WN, Knetsch CW, MacCannell DR, Harmanus C, Du T, Mulvey MR, et al. Development and Validation of an Internationally-Standardized, High-Resolution Capillary Gel-Based Electrophoresis PCR-Ribotyping Protocol for *Clostridium difficile*. PLoSOne. 2015;10(2):e0118150.

110. Eyre DW, Fawley WN, Best EL, Griffiths D, Stoesser NE, Crook DW, et al. Comparison of multilocus variable-number tandem-repeat analysis and whole-genome sequencing for investigation of *Clostridium difficile* transmission. J Clin Microbiol. 2013;51(12):4141-9.

111. Eyre DW, Walker AS. *Clostridium difficile* surveillance: harnessing new technologies to control transmission. Expert Rev Anti Infect Ther. 2013;11 (11):1193-205.

112. He M, Sebaihia M, Lawley TD, Stabler RA, Dawson LF, Martin MJ, et al. Evolutionary dynamics of *Clostridium difficile* over short and long time scales. Proc Natl Acad Sci U S A. 2010;107(16):7527-32.

113. Hyland M, Ofner-Agostini M, Miller M, Paton S, Gourdeau M, Ishak M, et al. N-CDAD in Canada: results of the Canadian Nosocomial Infection Surveillance Program 1997 N-CDAD Prevalence Surveillance Project. Can J Infect Dis. 2001;12(2):81-8.

114. Pepin J, Valiquette L, Alary ME, Villemure P, Pelletier A, Forget K, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ. 2004;171(5):466-72.

115. Pepin J, Valiquette L, Cossette B. Mortality attributable to nosocomial *Clostridium difficile*-associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ. 2005;173(9):1037-42.

116. McDonald LC, Killgore GE, Thompson A, Owens RC, Jr., Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med. 2005;353(23):2433-41.

117. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med. 2005;353(23):2442-9.

118. Beaujean DJ, Blok HE, Vandenbroucke-Grauls CM, Weersink AJ, Raymakers JA, Verhoef J. Surveillance of nosocomial infections in geriatric patients. J Hosp Infect. 1997;36(4):275-84.

119. Kuijper EJ, de WJ, Kato H, Kato N, van Dam AP, van der Vorm ER, et al. Nosocomial outbreak of *Clostridium difficile*-associated diarrhoea due to a clindamycin-resistant enterotoxin A-negative strain. Eur J Clin Microbiol Infect Dis. 2001;20(8):528-34.

120. van Dalen T, van Dijk Y, Kaan JA, Diepersloot RJ, Leguit P. *Clostridium difficile* outbreak in surgical wards. Ned Tijdschr Geneeskd. 1998;142(5):253-5.

121. Debast SB, Vaessen N, Choudry A, Wiegers-Ligtvoet EA, van den Berg RJ, Kuijper EJ. Successful combat of an outbreak due to *Clostridium difficile* PCR ribotype 027 and recognition of specific risk factors. Clin Microbiol Infect. 2009;15(5):427-34.

122. Goorhuis A, van der Kooi T, Vaessen N, Dekker FW, van den Berg R, Harmanus C, et al. Spread and epidemiology of *Clostridium difficile* polymerase chain reaction ribotype 027/toxinotype III in The Netherlands. Clin Infect Dis. 2007;45(6):695-703.

123. Kuijper EJ, van den Berg RJ, Debast S, Visser CE, Veenendaal D, Troelstra A, et al. *Clostridium difficile* ribotype 027, toxinotype III, the Netherlands. Emerg Infect Dis. 2006;12(5):827-30.

124. van den Hof S, van der Kooi T, van den Berg R, Kuijper EJ, Notermans DW. *Clostridium difficile* PCR ribotype 027 outbreaks in the Netherlands: recent surveillance data indicate that outbreaks are not easily controlled but interhospital transmission is limited. Euro Surveill. 2006;11(1):E060126.

125. van Steenbergen J, Debast S, E. vK, van den Berg R, Notermans D, Kuijper E. Isolation of *Clostridium difficile* ribotype 027, toxinotype III in the Netherlands after increase in *C. difficile*-associated diarrhoea. Euro Surveill. 2005;10(7):E050714.

126. Kuijper EJ, Barbut F, Brazier JS, Kleinkauf N, Eckmanns T, Lambert ML, et al. Update of *Clostridium difficile* infection due to PCR ribotype 027 in Europe, 2008. Euro Surveill. 2008;13(31).

127. Dingle KE, Didelot X, Quan TP, Eyre DW, Stoesser N, Golubchik T, et al. Effects of control interventions on *Clostridium difficile* infection in England: an observational study. Lancet Infect Dis. 2017.

128. European Centre for Disease Prevention and Control (ECDC). Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals. 2011-2012. Stockholm: ECDC; 2013.

129. Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, et al. Underdiagnosis of *Clostridium difficile* across Europe: the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID). The Lancet Infectious Diseases. 2014;14(12):1208-19.

130. U.S. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. 2013 4/23/2013.

131. Hunter JC, Mu Y, Dumyati GK, Farley MM, Winston LG, Johnston HL, et al. Burden of Nursing Home-Onset *Clostridium difficile* Infection in the United States: Estimates of Incidence and Patient Outcomes. Open forum infectious diseases. 2016;3(1).

132. European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European long-term care facilities. April–May 2013. Stockholm: ECDC; May 2014.

133. Bruijnesteijn van Coppenraet LE, Dullaert-de BM, Ruijs GJ, van der Reijden WA, van der Zanden AG, Weel JF, et al. Case-control comparison of bacterial and protozoan microorganisms associated with gastroenteritis: application of molecular detection. Clin Microbiol Infect. 2015;21(6):592e9-e19.

134. Khanna S, Pardi DS, Aronson SL, Kammer PP, Orenstein R, St Sauver JL, et al. The epidemiology of community-acquired *Clostridium difficile* infection: a population-based study. Am J Gastroenterol. 2012; 107(1):89-95.

135. Riley TV, Wymer V, Bamford VW, Bowman RA. *Clostridium-Difficile* in General-Practice and Community-Health. J Hyg (Lond). 1986;96(1):13-7.

136. Hirschhorn LR, Trnka Y, Onderdonk A, Lee ML, Platt R. Epidemiology of community-acquired *Clostridium difficile*-associated diarrhea. J Infect Dis. 1994;169(1):127-33.

137. Bloomfield LE, Riley TV. Epidemiology and Risk Factors for Community-Associated *Clostridium difficile* Infection: A Narrative Review. Infect Dis Ther. 2016;5(3):231-51.

138. Søes LM, Holt HM, Bottiger B, Nielsen HV, Andreasen V, Kemp M, et al. Risk factors for *Clostridium difficile* infection in the community: a case-control study in patients in general practice, Denmark, 2009-2011. Epidemiol Infect. 2014;142(7):1437-48.

139. Chitnis AS, Holzbauer SM, Belflower RM, Winston LG, Bamberg WM, Lyons C, et al. Epidemiology of community-associated *Clostridium difficile* infection, 2009 through 2011. JAMA InternMed. 2013; 173(14):1359-67.

140. Stoesser N, Eyre DW, Quan TP, Godwin H, Pill G, Mbuvi E, et al. Epidemiology of *Clostridium difficile* in infants in Oxfordshire, UK: Risk factors for colonization and carriage, and genetic overlap with regional *C. difficile* infection strains. PLoS One. 2017;12(8): e0182307.

141. Stone NE, Sidak-Loftis LC, Sahl JW, Vazquez AJ, Wiggins KB, Gillece JD, et al. More than 50% of *Clostridium difficile* Isolates from Pet Dogs in Flagstaff, USA, Carry Toxigenic Genotypes. PLoS One. 2016;11(10).

142. Knetsch C, Connor T, Mutreja A, S. vD, Sanders I, Browne H, et al. Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill. 2014;19(45).

143. Knight DR, Squire MM, Collins DA, Riley TV. Genome Analysis of *Clostridium difficile* PCR Ribotype 014 Lineage in Australian Pigs and Humans Reveals a Diverse Genetic Repertoire and Signatures of Long-Range Interspecies Transmission. Frontiers in microbiology. 2016;7:2138.

144. Hopman NE, Keessen EC, Harmanus C, Sanders IM, van Leengoed LA, Kuijper EJ, et al. Acquisition of *Clostridium difficile* by piglets. Vet Microbiol. 2011;149(1-2):186-92.

145. Keessen EC, Donswijk CJ, Hol SP, Harmanus C, Kuijper EJ, Lipman LJ. Aerial dissemination of *Clostridium difficile* on a pig farm and its environment. Environ Res. 2011;111(8):1027-32.

146. Keessen EC, Harmanus C, Dohmen W, Kuijper EJ, Lipman LJ. *Clostridium difficile* infection associated with pig farms. Emerg Infect Dis. 2013; 19(6):1032-4.

147. Anderson DJ, Rojas LF, Watson S, Knelson LP, Pruitt S, Lewis SS, et al. Identification of novel risk factors for community-acquired *Clostridium difficile* infection using spatial statistics and geographic information system analyses. PLoS One. 2017;12(5): e0176285.

148. Kwon JH, Lanzas C, Reske KA, Hink T, Seiler SM, Bommarito KM, et al. An Evaluation of Food as a Potential Source for *Clostridium difficile* Acquisition in Hospitalized Patients. Infect Control Hosp Epidemiol. 2016;37(12):1401-7.

149. Warriner K, Xu C, Habash M, Sultan S, Weese SJ. Dissemination of *Clostridium difficile* in food and the environment: Significant sources of *C. difficile* community-acquired infection? J Appl Microbiol. 2016.