Staphylococcus aureus colonization and infection: optimizing MRSA decolonization and addressing challenges in S. aureus bacteremia management

Westgeest, A.C.

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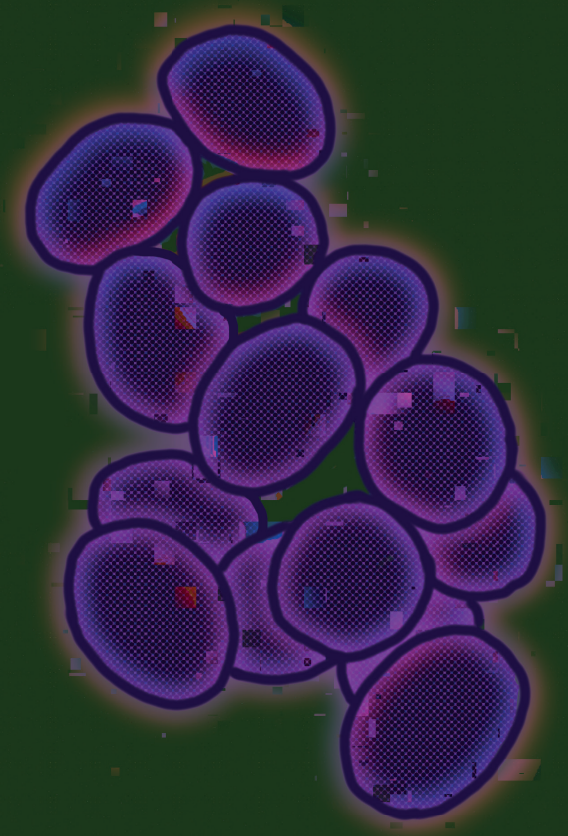

Annette Westgeest

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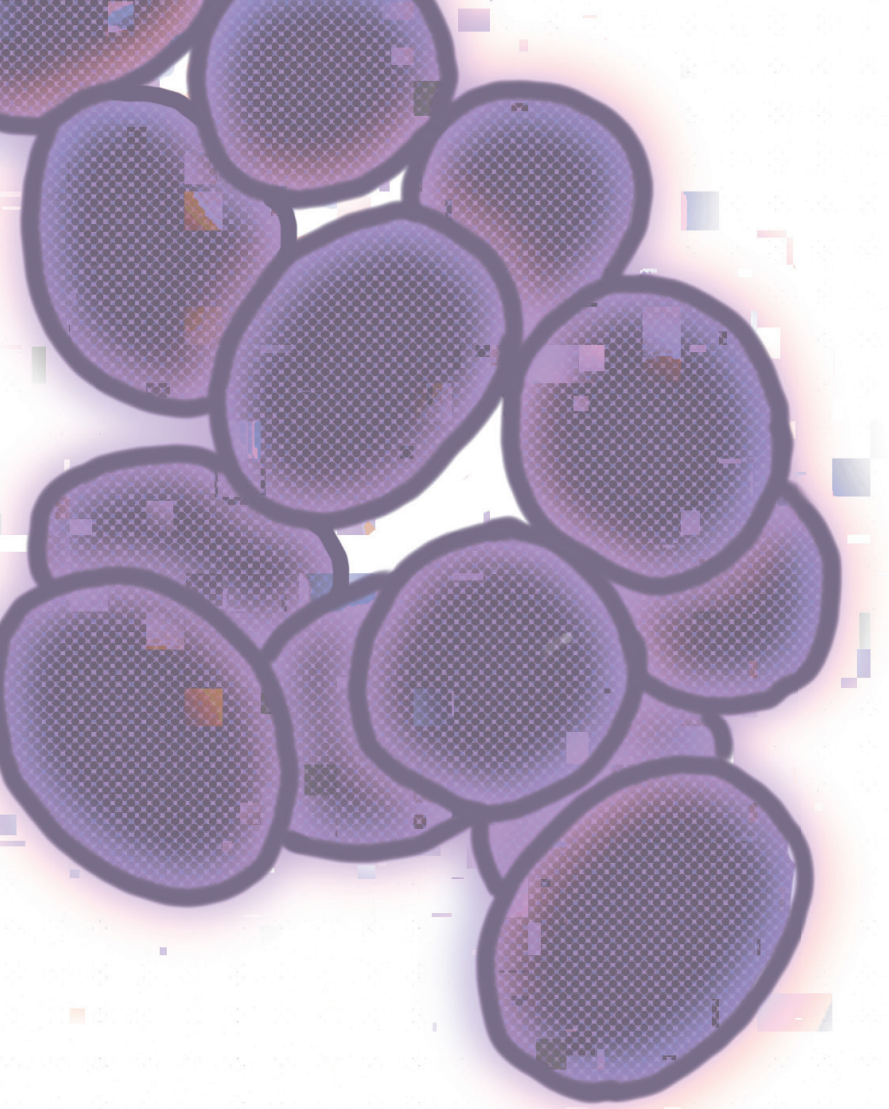


Chapter 1

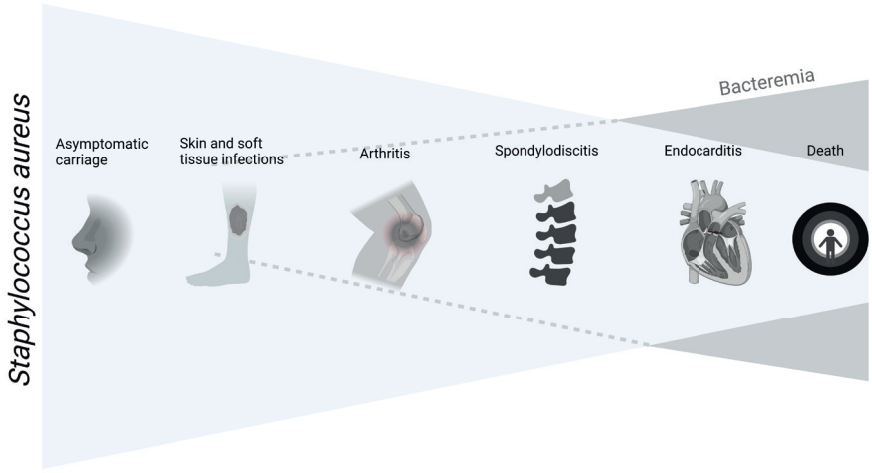
Introduction and outline
of the thesis

Introduction and outline of the thesis

Staphylococcus aureus is a fascinating pathogen. The Gram-positive spherically shaped bacterium is generally considered as the most virulent member of the *Staphylococcus* genus [1]. It adopted its name in the 1880s from the combination of the Greek words *staphyle* (bunch of grapes), *kokkos* (berry), and the Latin word *aureum* (gold), representing the appearance of the colonies on blood agar plates [2, 3].

As a human commensal, it colonizes more than half of the population, either intermittently or persistently [4]. Colonized persons are often asymptomatic and can be colonized in the anterior nares, throat, groin, skin, intestine, and other body sites. In only a minority, *S. aureus* causes disease – often caused by the individual's colonizing strain [5]. *S. aureus* is the causative agent of common and relatively benign infections such as folliculitis and impetigo. On the other end of the clinical spectrum, it is the causative agent of severe invasive infections such as endocarditis, spondylodiscitis, and bacteremia (Figure 1), and even the leading cause of mortality by bloodstream infections worldwide [6].

Figure 1. A glimpse of the spectrum of clinical manifestations of *Staphylococcus aureus*



The variability in both colonization and invasive infection of *S. aureus* is the result of a complex interplay between host, pathogen, and environment. Many aspects of these interactions are largely unexplained. Susceptibility of the host is, among other factors, influenced by age, immune response and genetic make-up. Although predisposing factors in the host have been identified, it remains impossible to predict who will be colonized, who will develop disease and in whom this disease will be severe.

Concerning the pathogen, *S. aureus* is capable of colonizing healthy individuals as well as causing catastrophic disease in many different animal hosts, including humans. It produces various virulence and immune evasion factors, interfering with the immune system of the host and preventing it from effectively warding off recurrent infections [7]. *S. aureus* has unique features, such as the ability to cause metastatic infections throughout the human body, mainly facilitated by the expression of surface proteins that mediate adhesion, and the tendency to persist in the bloodstream despite appropriate antibiotics. Besides, the pathogen has the ability to form biofilms leading to chronic device infections [8], and to produce multiple exotoxins, some of which are accountable for toxic shock syndrome and food poisoning [9]. Environmental factors are of influence on the variability of *S. aureus* as well, such as the prevalence in the community and the timely initiation of effective treatment.

A major additional complicating factor is the capacity of *S. aureus* to develop antimicrobial resistance.

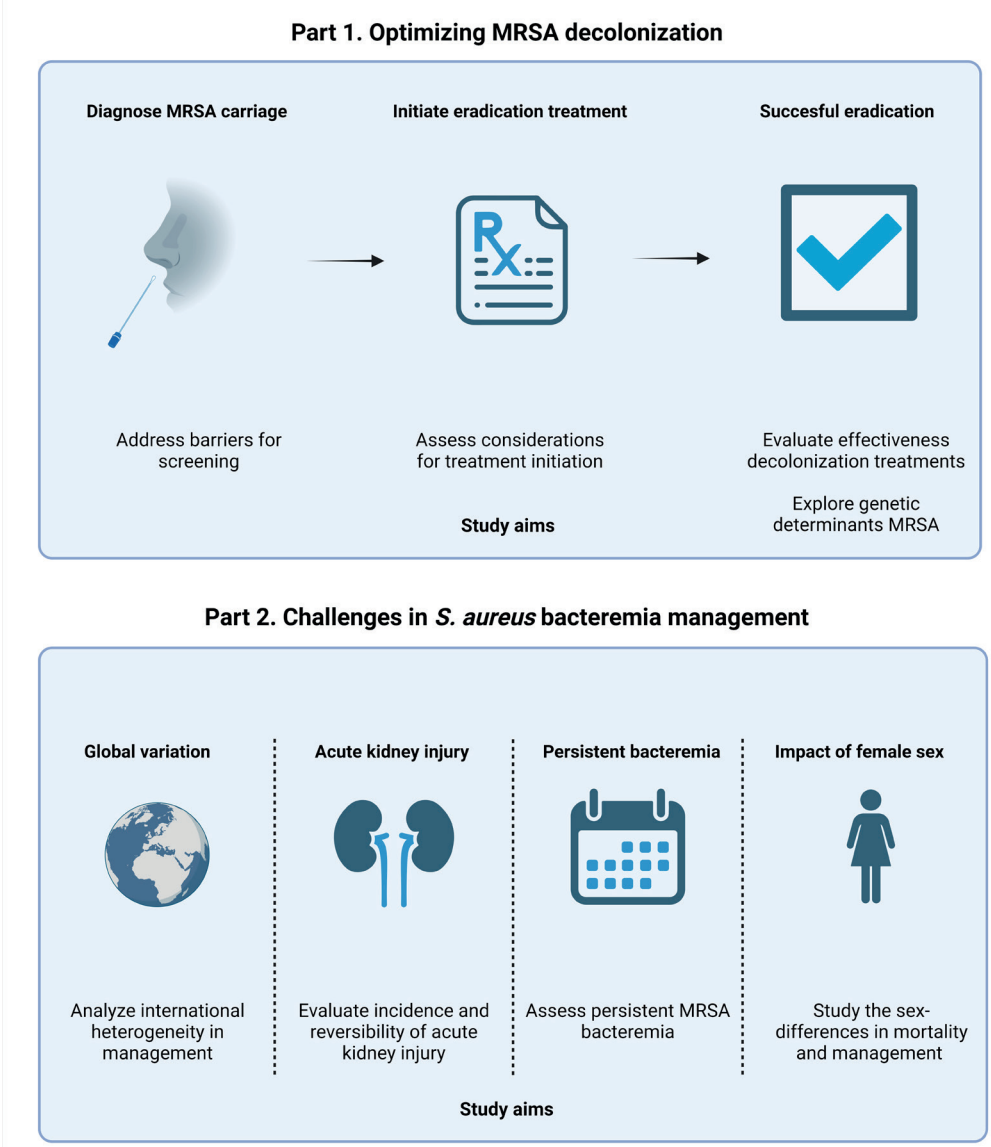
Antimicrobial resistance and *Staphylococcus aureus*

Antimicrobial resistance has significantly increased over the past decades, and is now in the top ten public health threats facing humanity, as declared by the World Health Organization (WHO) [10]. As a natural evolutionary response to antimicrobial exposure, bacteria develop resistance to antibiotics through multiple different mechanisms [11]. For *S. aureus*, the most relevant resistance mechanism is by acquiring a *mecA* gene through horizontal transfer of a mobile genetic element designated staphylococcal cassette chromosome *mec* (SCC*mec*), leading to methicillin resistance. The *mecA* gene encodes for a specific penicillin binding protein (PBP2a), which crosslinks bacterial peptidoglycans and has low affinity for beta-lactam antibiotics, causing resistance to almost all antibiotics within this class [12]. Methicillin-resistant *S. aureus* (MRSA) was first described in the early 1960s, shortly after the introduction of the antibiotic methicillin [13]. However, modern molecular phylogenetics suggest that MRSA emerged already by natural selection in the pre-antibiotic era and was further selected for by the widespread use of penicillin since the 1940s. Methicillin only provided better selective pressure for the bacterium to spread [14, 15].

Responsible for over 100,000 deaths in 2019, MRSA is currently the leading cause of mortality attributable to antimicrobial resistance in the world [16]. As a major actor in the field of antimicrobial resistance, MRSA also serves as an indicator for antimicrobial resistance in the global sustainable development goals of the United Nations [17].

Despite the high prevalence and global burden of *S. aureus*, many questions remain unanswered with respect to the management and risk factors of both colonization and invasive infection. Research is continuously ongoing in order to unravel the complexities of this extraordinary pathogen and the diseases it causes in humans. This thesis aims to address the optimization of MRSA decolonization and some of the frequently encountered challenges in *S. aureus* bacteremia management (Figure 2).

Figure 2. Graphical summary of thesis



Outline of the thesis

Optimization of MRSA decolonization

Colonization with *S. aureus* is a risk factor for developing subsequent infections. For bloodstream infections, this results from an endogenous infection source, reflected by identical isolates cultured from the blood and nares of patients with *S. aureus* bacteremia. Colonization with MRSA increases infection risk even more than colonization with methicillin-susceptible *S. aureus* (MSSA), in both patients and healthy individuals [18-21]. Decolonization therapy has been proven to reduce *S. aureus* infections, although the evidence for infection reduction outside of hospital settings is limited [22-24].

In the Netherlands, the MRSA prevalence is one of the lowest in the world [25]. This low prevalence is, next to the restricted use of antibiotics, to a large part ascribed to our 'search and destroy' policy [26, 27]. The policy consists of screening and preemptive isolation of patients at risk for MRSA carriership when hospitalized, and subsequent decolonization treatment when persistent carriership is found [28]. The aim of this policy is to minimize MRSA colonization in order to prevent transmission and infection.

The effectiveness of the 'search and destroy' policy depends on several consecutive steps. First of all, MRSA carriers need to be identified. The second step includes the initiation of eradication treatment. We evaluated barriers in these first steps of MRSA eradication care in **chapter 2**.

The third and final step involves the effectiveness of decolonization treatments, and is addressed in the next two chapters. Despite being notorious for nosocomial transmission and hospital outbreaks, MRSA with onset in the community has emerged over the past decades and has become endemic in large parts of the world [29, 30]. In **chapter 3**, we reviewed the evidence on individual decolonization strategies for MRSA, with particular emphasis on community-onset MRSA.

The Dutch guideline for MRSA eradication distinguishes between uncomplicated and complicated carriership [31]. Complicated carriership is defined as extra-nasal MRSA colonization, colonization with active skin lesions, foreign body material with connection to exterior, or previous failure of eradication treatment. Active skin lesions are recommended to be treated and foreign body material with connection to exterior to be removed before initiation of eradication treatment. Extra-nasal MRSA carriership is recommended to be treated with the combination of topical therapy and two systemic antimicrobial agents. However, which combination of systemic anti-staphylococcal antibiotics is most effective in MRSA eradication has not been clarified yet [32]. In **chapter 4**, the effectiveness of different MRSA decolonization

treatments for complicated MRSA carriage is analyzed.

Another potential influencing factor on effective decolonization is the genetic composition of the MRSA strain, as well as the host [33]. The complex genetic host-pathogen interaction in MRSA decolonization is relatively undiscovered, but is starting to gain interest as a result of the rapid developments in the field of molecular biology, especially whole genome sequencing. **Chapter 5** describes an explorative study on genomic characteristics of MRSA isolates that are associated with decolonization failure.

Challenges in *Staphylococcus aureus* bacteremia management

S. aureus bacteremia (caused by both MSSA and MRSA) is a highly variable disease affecting a heterogenous patient population. Consequently, the disease course varies greatly, ranging from transient uncomplicated bacteremia to disseminated infection, metastatic infections or persistent bacteremia despite appropriate antimicrobial therapy. All combined, the incidence of *S. aureus* bacteremia is estimated at 30 per 100,000 person years, and the overall 90-day mortality amounting to 20-30% [34, 35]. In the past decades the disease has been extensively studied, learning us that infectious disease consultation, follow-up blood cultures, and routine echocardiography all improve patients' outcomes [36, 37]. However, many challenges in the optimal management of *S. aureus* bacteremia remain. Different strategies are practiced throughout the world regarding optimal antibiotic regimen, oral switch therapy, treatment duration and defining persistence. **Chapter 6** describes the results of a survey of over 2,000 clinicians from 71 countries and 6 continents, about their treatment practices. It focuses on identifying global variation in management, diagnostics, and definitions of *S. aureus* bacteremia.

In clinical practice, a frequent complication in patients with *S. aureus* bacteremia is acute kidney injury. The complexity of this phenomenon lies in the combination of the diverse etiology – including prerenal, toxic/drug-related, immune-mediated, tubulointerstitial nephritis, and postrenal pathophysiology – and the lack of diagnostic tests to differentiate between them. Moreover, acute kidney injury has a significant impact on patient management and outcome [38]. Still, knowledge on acute kidney injury in *S. aureus* bacteremia is limited. In **chapter 7**, we evaluated the incidence, reversibility and risk factors for the development of acute kidney injury in patients with *S. aureus* bacteremia.

As mentioned before, *S. aureus* has the ability to persist in the bloodstream despite adequate antimicrobial treatment. Persistent bacteremia has been associated with increased mortality compared to those whose bacteremia promptly resolves [39, 40].

Although very rare in countries with low MRSA prevalence such as the Netherlands, persistent MRSA bacteremia is relatively common in the United States [41]. A variety of host and pathogen factors are potentially associated with persistence, and few alternative therapeutical options for persistent bacteremia have gradually evolved over time. We reviewed the literature on persistent MRSA bacteremia in **chapter 8**.

S. aureus bacteremia affects both males and females around the globe. Females have a lower *a priori* risk of acquiring *S. aureus* bacteremia compared to males, and represent approximately 40% of the *S. aureus* bacteremia population [42]. Although less frequently affected, some previous studies reported an increased mortality risk of up to 30% in females with *S. aureus* bacteremia as compared to males [43, 44]. However, other studies did not find a sex inequality in mortality, or even a higher mortality in males in a subgroup of patients with more comorbidities [45, 46]. Thus, the impact of female sex on outcome among patients with *S. aureus* bacteremia remained unclear. **Chapter 9** describes our study on sex-differences in mortality, patient characteristics, disease aspects and management, in a large cohort of over 3,000 *S. aureus* bacteremia patients. In **chapter 10**, a systematic review and meta-analysis was conducted to determine the true association of female sex and mortality in *S. aureus* bacteremia.

The results of this thesis are summarized and discussed in **chapter 11**.

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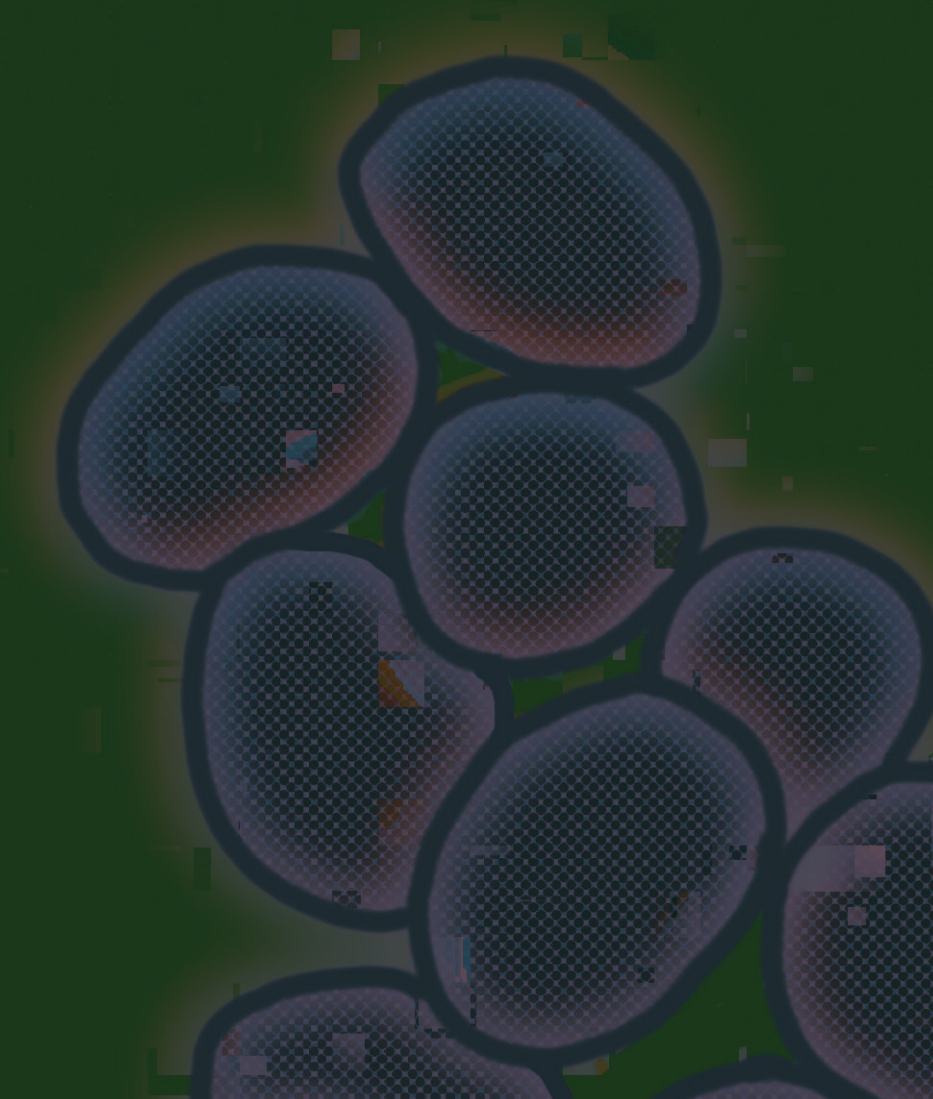
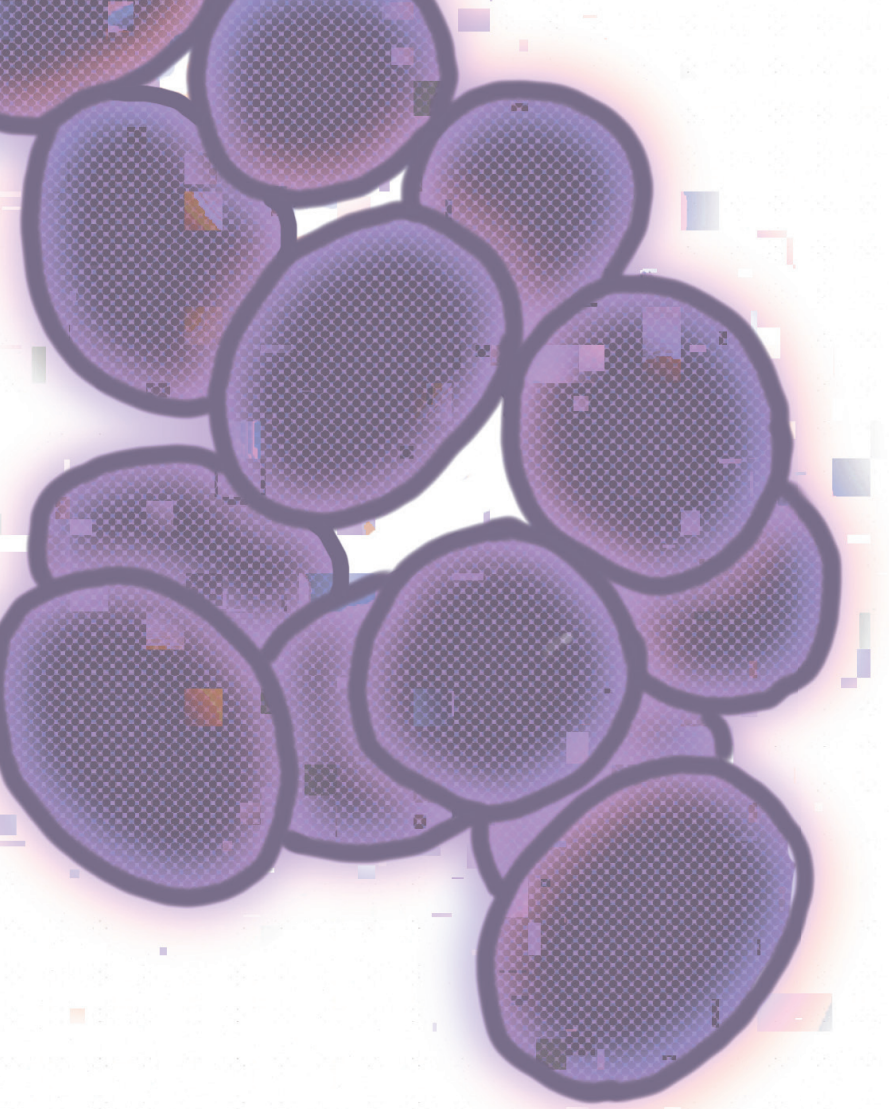
Part I

Optimization of MRSA decolonization





Chapter 2

Exploring the barriers in the uptake of the Dutch MRSA ‘search and destroy’ policy using the cascade of care approach

Annette C. Westgeest, Emile F. Schippers, Martijn Sijbom, Leo G. Visser, Mark G. J. de Boer, Mattijs E. Numans, Merel M. C. Lambregts, on behalf of the MRSA Network Holland West

Antibiotics. 2022 Sep 8;11(9):1216.

Abstract

The Dutch 'search and destroy' policy consists of screening patients with an increased risk of methicillin-resistant *Staphylococcus aureus* (MRSA) carriership and subsequent decolonization treatment when carriership is found. Decolonization therapy of individual MRSA carriers is effective. However, the effectiveness of the national 'search and destroy' policy is dependent on the entire cascade of care, including identification, referral, and subsequent treatment initiation in MRSA carriers. The aim of this study was to evaluate the leakages in the cascade of MRSA decolonization care. We assessed familiarity with the 'search and destroy' policy and the barriers in the uptake of MRSA eradication care using a questionnaire among 114 Dutch general practitioners. The main reasons for treatment were planned hospital visits, occupational reasons, and infections. The main reasons for refraining from eradication treatment were unfamiliarity with the 'search and destroy' policy and the assumption that MRSA carriership is often self-limiting. To optimize the continuity of the cascade of care, interventions should be aimed at supporting general practitioners and facilitating treatment and referral.

Introduction

Antimicrobial resistance is a global health threat that causes millions of deaths [1]. The WHO has declared that antimicrobial resistance is one of the top ten global public health threats facing humanity [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major actor in the field of antimicrobial resistance. In 2019, 100.000 deaths and 3.5 million disability-adjusted life-years (DALYs) were attributable to infections with MRSA [3]. Colonization with MRSA leads to increased infection rates of up to 25% [4–6].

Colonization and infection rates are known to vary throughout the world. Historically, in the Netherlands, MRSA infection rates are low. Less than 5% of invasive *Staphylococcus aureus* isolates are resistant to methicillin. Together with the Nordic European countries, the Dutch prevalence of MRSA is the lowest in the world [7]. The estimated nasal colonization rate in the Dutch population is 0.03–0.17%, compared to 0.9–1.5% in the US [8].

The healthcare system in the Netherlands has executed a national 'search and destroy' policy since 1988, which is outlined in the guidelines of the Dutch Working Party on Infection Prevention (WIP) [9]. The policy consists of the screening and preemptive isolation of patients with an increased risk of MRSA carriership when hospitalized

and subsequent decolonization treatment when persistent carriership is found [10–12]. Examples of an increased risk are preceding events such as hospitalization in a country where MRSA is endemic, or a confirmed MRSA-carrying household contact. The aim of the policy, which is endorsed by the Dutch health council, is to keep the MRSA prevalence and the associated disease burden low [13]. Cost-effectiveness was confirmed in the years thereafter, with an estimated saving of up to EUR 400 per hospital per year [10,14].

As part of this 'search and destroy' policy, decolonization treatment in MRSA carriers has proven to be an effective preventive strategy in reducing infection and hospitalization rates [15]. The success rate of decolonization treatment, defined as three consecutive negative MRSA swabs from nose, throat, and perineum, is as high as 86% [16]. However, the effectiveness of the policy is also dependent on the initial identification of carriership and the initiation of treatment.

Therefore, the effectiveness of the national policy relies on the correct execution of several consecutive steps in a so-called cascade of care and involves several healthcare professionals. In HIV care, a similar approach was taken and led to the clarification of the culprits in the uptake of combination anti-retroviral therapy (cART) [17]. Following this example, this approach was applied to tuberculosis and hepatitis C [18,19]. We hypothesize that the same approach is applicable to MRSA decolonization care as well (Figure 1). Within the MRSA decolonization cascade of care, individuals may be lost, which is referred to as leakage, and is analogous to the cART roll-out strategies. Understanding at which steps this leakage occurs will provide information to optimize MRSA eradication strategies [20].

The aim of our current study was to evaluate the leakages within the cascade of MRSA decolonization care and the main reasons for them. We carried out a questionnaire study amongst general practitioners (GPs) to gain insight into their familiarity with the 'search and destroy' policy and to evaluate barriers in the uptake of MRSA eradication care. The knowledge generated will help to determine specific targets that can be addressed to keep MRSA prevalence low and to contribute to a reduced burden of antimicrobial resistance.

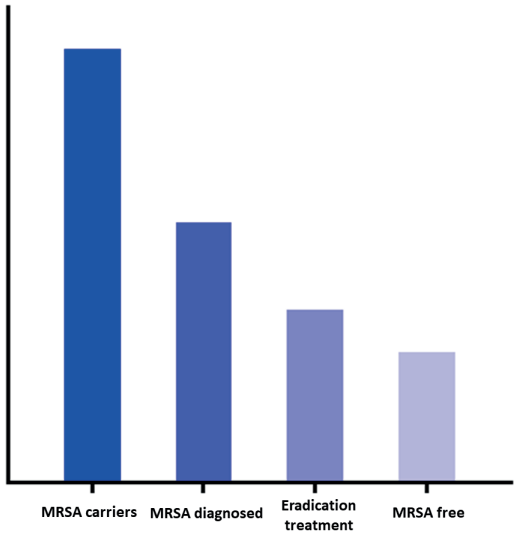


Figure 1. Conceptual graphic of the cascade of care in MRSA decolonization. Legend: The first column addresses the total number of MRSA carriers in the Netherlands. The second column represents the proportion of carriers that is diagnosed. The third column addresses the MRSA carriers that are diagnosed and undergo eradication treatment. The last column represents the success rate of complicated MRSA eradication treatment. In every step of this conceptual cascade of care, there is the potential for leakage. As this figure represents a conceptual model, the columns are not quantified.

Methods

The questionnaire study was executed in primary care as GPs hold a central position in the Dutch healthcare system. All Dutch citizens are registered with a general practitioner (GP), who is the first point of contact in case of illness and acts as a gatekeeper to secondary care. With regard to MRSA carriership, the GPs are often the first healthcare professionals to be in contact with patients at risk or to detect MRSA carriership.

Questionnaire development and distribution

The regional MRSA Network developed a questionnaire that was reviewed by a panel consisting of a general practice specialist and an infectious disease specialist (Supplementary File S1). The questionnaire included 14 questions on the 'search and destroy' policy, the screening of risk patients, the difference between complicated

and uncomplicated carriership, and eradication therapy. Two case vignettes were included to assess daily practice (Box 1). The target population consisted of GPs in the Netherlands. The questionnaire was hosted on Formdesk, a web-based survey platform, and was distributed via different networks of GPs and newsletters from participating hospitals. The majority of the recipients were situated in the western part of the Netherlands. There was the possibility of responding anonymously. The questionnaire was accessible between 7 March 2022 and 13 June 2022. Descriptive statistics were used to summarize the data derived from the Formdesk software.

Case A: A 26 years-old healthy male was admitted in the hospital during a holiday in Spain because of a trauma. After returning in the Netherlands, you perform culture swabs from, throat and perineum. The nasal culture is positive for MRSA. There are no skin lesions. There are no hospital visits planned.

Case B: A 56 years-old male with a history of heart failure and chronic kidney disease, was screened for MRSA carriership by you following a hospital admission. He is MRSA positive in nose, throat and perineum.

Box 1. Case vignettes.

Legend: Two clinical case vignettes were included in the questionnaire. Case A describes a patient with uncomplicated carriership. Case B describes a patient with complicated carriership. The guideline recommends treatment with topical therapy in case A and treatment with additional (systemic) antibiotics in case B.

Definitions

The Dutch national guideline on the treatment of MRSA carriers recommends different eradication treatments depending on the type of carriership. Uncomplicated MRSA carriership is defined as having all of the following features: (i) the presence of MRSA exclusively located in the nose, (ii) no active infection with MRSA, (iii) in vitro sensitivity for mupirocin, (iv) the absence of active skin lesions, (v) the absence of foreign material that connects an internal body site with the outside (e.g., urine catheter or external fixation material), and (vi) no previous failure of decolonization treatment. All other cases are considered to be complicated colonization [21]. Uncomplicated carriership is treated with topical therapy (mupirocin topically applied to the nares and disinfecting shampoo) and hygienic measures. In the case of complicated MRSA carriage, additional systemic antimicrobial therapy with a combination of two antibiotic agents is recommended. Furthermore, the guideline recommends the screening of household contacts (and sometimes pets) and the simultaneous treatment of colonized household contacts [21].

Results

The questionnaire was completed by 114 Dutch GPs. The majority of the GPs (98/114, 86%) performed screening for MRSA carriership. Recent admission to a hospital abroad was more often considered to be the reason for screening in older patients with comorbidity (89/114, 78%) compared to younger patients without comorbidity (77/114, 68%). A previous infection with MRSA was considered to be a reason for screening by 55/114 (48%) of the GPs and a positive household contact by 39/114 (34%) of the GPs.

The majority of the respondents, 98/114 (86%), reported having 1-3 new MRSA cases per year. Fifteen GPs (15/114, 13%) stated that they had never had a single patient in his/her practice. The median prevalence of MRSA carriers per practice was 2 (interquartile range 0–4). With regard to the familiarity with the explicit 'search and destroy' policy in the Netherlands, 98/114 (86%) of the GPs indicated that they were not familiar with this policy.

Initiation of eradication therapy and/or referral for treatment

Almost half of the GPs (52/114, 46%) estimated that <20% of the MRSA carriers in their practice received eradication therapy. With respect to the indication for eradication treatment, most of the GPs (58/114, 51%) stated that only specific MRSA carriers should be eligible for eradication treatment, namely if there is a specific reason (e.g., frequent hospital visits) (58/58, 100%), if the patient is a healthcare worker with clinical duties (52/58, 90%), if the patient has an infection with MSRA (42/58, 72%), or if the patient insists on treatment (10/58, 17%).

The most important reasons to refrain from eradication therapy were: the potentially self-limiting nature of MRSA carriership (59%), unfamiliarity with the Dutch 'search and destroy' policy (25%), the burden of treatment for the patient (23%), the lack of any recommendation being known GP protocols (18%) and the patients' explicit request not to be treated (18%) (Table 1).

Table 1. The attitude of GPs towards indication for treatment of MRSA carriership.

	Frequency n/n (%)
Indication for eradication treatment	
In all MRSA carriers	18/114 (16)
In selected cases	58/114 (51)
Planned/expected hospital visits	*58/58 (100)*
Infections with MRSA	*42/58 (72)*
Occupational reason (e.g., healthcare worker)	*52/58 (90)*
Patients' request	*10/58 (17)*
In none of the MRSA carriers	1/114 (1)
Unknown	37/114 (32)
Reasons to refrain from treatment *	
Potential self-limiting nature of MRSA carriership	57/96 ** (59)
Unfamiliarity with the policy	24/96 (25)
Treatment burden for patients	22/96 (23)
Lack of recommendation in the GP guideline	17/96 (18)
Patients' request	17/96 (18)
Absence of benefit for the patient	11/96 (11)
Sense of incompetence to guide a treatment	10/96 (10)
Absence of benefit for the society	5/96 (5)
Costs for the patient	4/96 (4)
Other ***	19/96 (20)

Legend: Indications for MRSA eradication according to Dutch general practitioners and reasons not to initiate treatment or refer for treatment. * Multiple answers possible. ** Eighteen GPs who answered in the previous question that all MRSA carriers have an indication for eradication treatment were not asked for reasons to refrain from treatment. *** Other reasons mentioned in free text: not a task for the GP, assumption of no curation, never considered, patient in palliative setting. GP = general practitioner.

Forty-four respondents (44/114, 39%) had treated patients with (complicated or uncomplicated) MRSA carriership themselves—in all cases or in selected cases. When treating a patient for MRSA carriership, 10/44 (23%) of the responding GPs included the screening and treatment of household contacts in the initial treatment attempt, 5/44 (11%) included the household contacts only after a failed treatment attempt, and 12/44 (27%) never included household contacts. Other GPs (17/44, 39%) stated that they asked an expert for advice. The most important reasons to refrain from referring an MRSA carrier to the hospital were unfamiliarity with the existence of MRSA outpatient clinics (55/114, 48%), feeling competent in the self-performance of treatment (19/114, 17%), and the absence of this recommendation in the guideline (17/114, 15%) (Table 2).

Table 2. Treatment of MRSA carriers.

	Frequency n/n (%)
Estimated proportion of carriers in a GP practice that receive treatment *	
<20%	52/114 (46)
20–40%	8/114 (7)
40–60%	11/114 (10)
60–80%	12/114 (11)
80–100%	25/114 (22)
Unknown	6/114 (5)
Treatment by GP or referral to hospital	
Treatment by GP in all cases	12/114 (11)
Referral to a hospital in all cases	40/114 (35)
Treatment by GP in selected cases	32/114 (28)
Uncomplicated carriership	*23/32 (72)*
Patient preference for GP treatment	*9/32 (28)*
Other	*8/32 (25)*
None of the above	27/114 (24)
Reasons not to refer to a hospital **	
Unfamiliar with the existence of MRSA outpatient clinics	55/114 (48)
Competent in self-performance	19/114 (17)
Lack of recommendation in GP protocol	17/114 (15)
Patients' request not to be referred	13/114 (11)
Costs for the patient ***	13/114 (11)
Administrative burden of a referral	3/114 (3)
Other ****	33/114 (29)
Unknown	10/114 (9)

Legend: * Estimation of the proportion of known MRSA carriers in the practice that are receiving eradication therapy or have received eradication treatment in the past. ** Multiple answers possible. *** In the Netherlands, the health insurance charges the patient an obligatory deductible excess for hospital care. **** Other reasons mentioned in free text were: consultation of specialist is sufficient, never considered, palliative settings, refusal of hospital, or not specified. GP = general practitioner.

Two cases were presented in the questionnaire: case A was the description of a young patient with an uncomplicated carriership, and case B was a case of a complicated carriership (Box 1). Of the respondents, 40/114 (35%) were aware of the difference between 'complicated' versus 'uncomplicated' MRSA colonization. Respectively, 37 (33%) and 3 (3%) of the GPs would refrain from treatment in case A and B, 15 (13%) and 56 (49%) would refer the patient to a hospital for treatment, and 29 (25%) and 31 (27%) would first consult a specialist. Of the GPs that would initiate treatment in these cases themselves (17 in case A and 14 in case B), the treatment prescription was in accordance with the treatment guideline for 12/17 (71%) in case A (uncomplicated carriership) and for 8/14 (57%) in case B (complicated carriership). In both cases, four

GPs (24%, 29%) indicated to add or refrain from systemic antibiotics where this was not in accordance with the guideline (Supplementary File S2).

Discussion

The main finding of this study is that there is significant leakage in the cascade of MRSA decolonization care. Firstly, the vast majority of the responding GPs are not familiar with the explicit 'search and destroy' policy. Secondly, when evaluating a patient with MRSA carriage, many assumptions are made to refrain from eradication treatment. Thirdly, eradication treatment is not always in accordance with the guideline. The conceptual steps of the cascade of MRSA colonization care are visualized in Figure 1.

For optimal effect of the strategy, adherence to each consecutive step is crucial. Based on our findings, the uptake of decolonization care in the Netherlands, as part of the 'search and destroy' policy, is not flawless. All subsequent process steps in the cascade have the potential for improvement. We summarized the main leakages of the cascade and the possible solutions in Table 3. The most apparent opportunity for the improvement of its implementation is through expanding familiarity with the 'search and destroy' policy. All three steps in the cascade could benefit from the training/education of both the patients and the professionals. In addition, incorporating the policy in the GP practice guidelines should be considered in order to support the entire process from screening to successful eradication. The current national MRSA decolonization guideline is primarily targeted at medical specialists, and the recommendations for screening and treatment have not yet been translated to the Dutch GP guidelines [22]. At the patient level, financial barriers exist that could be targeted by waving the excess fee for MRSA decolonization care.

Despite the described leakages in the identification and treatment of MRSA carriership, the MRSA prevalence is low in our country compared to surrounding countries. The estimated nasal colonization rate in the Netherlands was 0.03–0.17% in 2010–2017 [23]. It is generally accepted that this is largely attributed to the 'search and destroy' policy [11,24-27]. The policy seems to be effective, despite the leakages we found in the decolonization cascade. The effectivity of the policy as a whole is only partly determined by the uptake of screening and decolonization therapy. Another important arm of the 'search and destroy' policy—the preemptive isolation of patients at risk—was not assessed in the current study. There has been debate about the rigorous 'search and destroy' policy in the past. Up to the present day, it is the subject of discussion whether healthy carriers that do not have any connections with hospital healthcare should be treated [21]. This is reflected in our results, where the GPs were less inclined to treat a young healthy MRSA carrier compared to an older patient with comorbidity. Although this is a leak in the cascade of care, not

treating this subset of MRSA carriers is justifiable as stated in the Dutch guideline.

Overall, the last report of the Dutch health council to the Ministry of Health in 2006, advising the continuation of the 'search and destroy' policy, is still valid [13]. Efficacy and cost-effectiveness have been demonstrated in the past [10,14]. The semi recent history of the United Kingdom is an extra confirmation of the effectiveness of this approach. In the UK, a similar strict MRSA policy was carried out in the 1980s. After the policy was tempered in the 1990s, the percentage of methicillin resistance in *Staphylococcus aureus* bacteremia increased steeply from <2% to >30% [28,29]. This percentage is now lower due to rigorous measures on hygiene and the mandatory reporting of MRSA, as part of a major public health infection prevention campaign [30].

To our knowledge, this study is the first to map the MRSA cascade of care. Although the methodology does not enable the quantification of the leakage within the different cascade steps, it does provide specific targets for the optimization of the cascade. The central position of GPs in the healthcare system is a characteristic of the Netherlands. However, the targets for optimization and proposed interventions could be translated to settings where GPs do not hold a central position, with a greater focus on hospitals.

A limitation of the study is the fact that all results were self-reported. Answers are subject to bias, and potential targets may have been missed. Furthermore, the majority of the respondents were from one region in the Netherlands, which is mainly an urbanized area. In regions with more agriculture and more livestock-associated MRSA, knowledge about MRSA and attitudes towards MRSA carriership may differ [31]. Another limitation is the fact that the response rate was unknown as a result of the various ways (e.g., newsletters) that the questionnaire was distributed. Assuming that the GPs with an affinity with MRSA were more inclined to respond, bias would be in favor of an overall knowledge of the policy. We believe that the identified barriers are valid, even if the response rate were to be relatively low.

Conclusions

In conclusion, the results of this survey and the derived cascade of care reveal that there are barriers in the uptake of the 'search and destroy' MRSA policy in the Netherlands. Low health-provider familiarity with the policy, lack of GP guidelines on the topic, and financial constraints are key factors. To optimize the continuity of the cascade of care, interventions should be aimed at supporting healthcare professionals in the execution of the 'search and destroy' policy. Eventually, this will be beneficial both on the population level and for the individual patient.

Table 3. Leakages in cascade of MRSA decolonization care and possible solutions.

Cascade Leakage	Causes	Potential Interventions
From colonization to diagnosis	Unfamiliarity with the 'search and destroy' policy	• Education of patients and the public • Education of healthcare professionals • Accessible information on MRSA policy
	Unfamiliarity with screening indications	• Education of healthcare professionals • Incorporate screening advice in culture results that are provided by microbiology lab (in case of MRSA infection)
	Financial burden associated with screening (excess fee)	• Exempt screening costs from the patients' obligatory deductible excess fee
From diagnosis to initiation of treatment	Unfamiliarity of GPs with the existence of MRSA outpatient clinics	• Promote MRSA outpatient clinics through newsletters and by incorporating referral details in culture results
	Lack of indications for decolonization treatment in the GP protocol	• Include a paragraph on eradication treatment in the GP skin/soft tissue infection guideline
	Perceived incompetence to start treatment by GP	• Facilitate easy consultation with microbiologist or infectiologist • Incorporate treatment protocol in the GP guideline
	Patients' request to refrain from treatment	• Patient education (e.g., website and/or patient information folders)
	Financial burden associated with treatment/referral (excess fee)	• Exempt treatment/referral costs from the obligatory deductible excess fee
From treatment to successful eradication	Knowledge deficit, e.g., different treatment of complicated and uncomplicated carriership	• Education of healthcare professionals • Facilitate easy consultation with an infectiologist or microbiologist • Provide patient instruction materials
	Lack of treatment protocol in GP guidelines	• Incorporate treatment protocol in GP guidelines

Legend: Causes of leakages in the cascade of MRSA decolonization care derived from the questionnaire and possible solutions devised by the MRSA Network. GP = general practitioner.

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Appendix A. Questionnaire

The original questionnaire was in Dutch. For publication purposes, it was translated to English.

1. Do you ever have patients with a positive MRSA culture?

   Never

   Less than once a year

   1-3 times a year

   More than 3 times a year

2. How many patients from your practice are proven MRSA carrier at this moment? (estimation)

   .....patients

3. What proportion of the MRSA carriers from your practice are treated for MRSA carriership now or in the past? (estimation)

   80-100%

   60-80%

   40-60%

   20-40%

   20%

4. Are you familiar with the difference between complicated and uncomplicated MRSA carriership?

   Yes / No

5. Do you ever screen patients for MRSA carriership?

   No, never

   Yes, if: .....

6. Which of the following patients would you screen for MRSA carriership? (multiple answers possible)

   27 years-old healthy male who was hospitalized for 3 days in Spain because of a trauma

   20 years-old student who has a MRSA positive household contact

   60 years-old male who was admitted at the ICU in Spain because of a myocardial infarction

   40 years-old female who had a MRSA cultured from a furuncle one month ago

   None of the above

7. In your opinion, when does a patient who is MRSA carrier qualify for eradication therapy?

   Always

   Sometimes, namely in case of: (multiple answers possible)

   - o A specific reason, e.g. when frequent hospital visits are expected
   - o The patient is suffering from MRSA infections
   - o The patient is a health care worker with clinical duties
   - o The patients insists
   - o Other: .....

   Never

   I do not know

8. If not always, what are reasons for you to refrain from MRSA eradication treatment? (multiple answers possible)

   It is not in the GP guideline

   I was not familiar with the recommendation of eradication of MRSA carriership

   In my opinion it is not useful for the patient

   In my opinion it is not useful for the society

   The costs (own risk) for the patient

   Patients do not wish to be treated

   MRSA carriership can resolve on its own

   The eradication treatment is too much of a burden for the patient

   I do not feel competent to guide a MRSA eradication treatment

   Other: .....

The following questions are about treating yourself or referring to a MRSA outpatient clinic. These are outpatient clinics where MRSA patients are treated by an infectiologist/microbiologist.

9. Do you perform eradication therapy of MRSA carriers yourself?

   I always treat myself, I never refer to a hospital.

   I always refer to a hospital, I never perform MRSA eradication myself.

   I treat some patients myself and refer other patients to the hospital.

   I never perform this treatment and never refer to a hospital either.

10. If option 3 at question 9: which patients do you treat yourself?

    - o Patients with uncomplicated carriership
    - o Patients who do not want to be referred to an outpatient clinic

- Patients who still need to pay their own risk (health insurance)
- Other

11. What are reasons for you to refrain from referral of patients with MRSA carriership? (Multiple answers possible)

    I did not know of the existence of MRSA outpatient clinics

    It is not recommended in the GP protocol to refer patients for eradication

    I feel competent in performing the treatment myself

    Patients do not wish to be referred

    The costs for the patient

    The administrative burden that comes with a referral

    Other: .....

12. Are you familiar with the 'search and destroy' policy with regards to MRSA? This 'search and destroy' policy means patients with high risk of MRSA colonization need to be screened and that we aim at eradication treatment of MRSA carriers.

    Yes / No

Now we want to present two cases.

13. Case A: A 26 years-old healthy male was admitted in the hospital during a holiday in Spain because of a trauma. After returning in the Netherlands, you perform culture swabs from nose, throat and perineum. The nasal culture is positive for MRSA. There are no skin lesions. There are no hospital visits planned.

What do you recommend with regards to the MRSA carriership?

No eradication treatment

I treat the MRSA carriership myself

I refer the patient to the outpatient clinic

Other: .....

In case of treatment yourself, which treatment do you prescribe?

Mupirocin nose cream + disinfecting soap + hygienic measures

The above in combination with systemic antibiotics

Other: .....

14. Case B: A 56 years-old male with a history of heart failure and chronic kidney disease, was screened for MRSA carriership by you following a hospital admission. He is MRSA positive in nose, throat and perineum. What do you recommend with regards to the MRSA carriership?

    No eradication treatment

    I treat the MRSA carriership myself

    I refer the patient to the outpatient clinic

    Other: .....

In case of treatment yourself, which treatment do you prescribe?

Mupirocin nose cream + disinfecting soap + hygienic measures

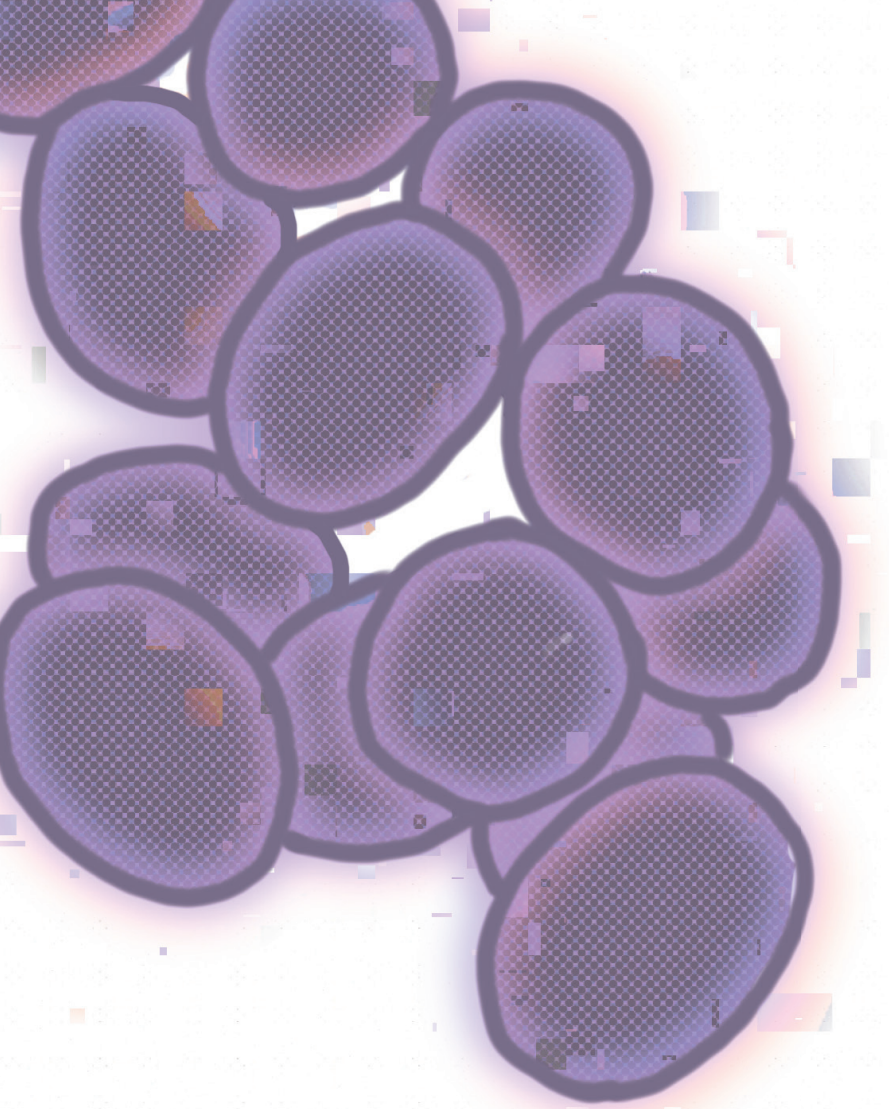
The above in combination with systemic antibiotics

Other: .....

Appendix B. GP's responses to clinical cases

	Frequency n/n (%)
Case A. Treatment of uncomplicated carriership	
No eradication treatment	37/114 (33)
Treatment by GP	17/114 (15)
Topical therapy + hygienic measures	*12/17 (71)*
Topical therapy + hygienic measures + systemic therapy	*4/17 (24)*
Other	*1/17 (6)*
Referral to hospital/MRSA clinic	15/114 (13)
Consultation with specialist	29/114 (25)
Other	15/114 (13)
Case B. Treatment of complicated carriership	
No eradication treatment	3/114 (3)
Treatment by GP	14/114 (12)
Topical therapy + hygienic measures	*4/14 (29)*
Topical therapy + hygienic measures + systemic therapy	*8/14 (57)*
Other	*2/14 (14)*
Referral to hospital/MRSA clinic	56/114 (49)
Consultation with specialist	31/114 (27)
Other	10/114 (9)

Legend. Case A. *A 26 years-old healthy male was admitted in the hospital during a holiday in Spain because of a trauma. After returning in the Netherlands, you perform culture swabs from nose, throat and perineum. The nasal culture is positive for MRSA. There are no skin lesions. There are no hospital visits planned. Case B. A 56 years-old male with a history of heart failure and chronic kidney disease, was screened for MRSA carriership by you following a hospital admission. He is MRSA positive in nose, throat and perineum.*



Chapter 3

Eradication of community-onset MRSA carriage: a narrative review

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization increases infection risk in both patients and healthy individuals. Decolonization therapy has been proven to reduce *S. aureus* infections, but data on the effectiveness of individual decolonization strategies in community-onset MRSA carriage are scarce.

Objectives

The aim of this narrative review was to summarize the evidence on strategies for the elimination of MRSA colonization in community-onset MRSA carriers.

Sources

PubMed database was searched for studies on MRSA eradication, from inception to July 2023. *Content:* Topical therapy is proven to be effective in nasal-only carriage and in temporary load reduction. Mupirocin nasal ointment in combination with chlorhexidine body wash is highly effective in nasal-only MRSA carriers in the community as well. In patients with extra-nasal colonization, addition of orally administered antibiotics likely increases success rates compared with topical therapy alone. Studies on systemic treatment of extra-nasal MRSA decolonization are subject to a high heterogeneity of antimicrobial agents, treatment duration, and control groups. The majority of evidence supports the use of a combination of topical therapy with rifampin and another antimicrobial agent. Decolonization treatment with probiotics is a promising novel non-antibiotic strategy. However, achieving long-term decolonization is more likely in countries with low MRSA prevalence, given the risk of recolonization in a context of high MRSA prevalence.

Implications

The decision to pursue community-onset MRSA eradication treatment in the individual patient should be based on the combination of the treatment objective (short-term bacterial load reduction in health care settings vs. long-term eradication in community settings), and the likelihood of successful decolonization. The latter is influenced by both individual risk factors for treatment failure, and the risk of recolonization. The addition of a combination of systemic antibiotics is rational for extra-nasal long-term decolonization. To determine the most effective systemic antimicrobial agents in MRSA decolonization, more research is needed.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of mortality attributable to antimicrobial resistance [1]. The pathogen is notorious for its nosocomial transmission and hospital outbreaks. On top of that, community-onset MRSA (CO-MRSA) has emerged over the past decades and has become endemic in large parts of the world [2]. Although often carried asymptomatically in the anterior nares, skin lesions, and elsewhere, *S. aureus* is an important cause of severe infections such as bacteraemia. Isolates cultured from blood and the nares are identical in the large majority of patients with *S. aureus* bacteraemia, suggesting an endogenous infection route [3]. Colonization with MRSA increases infection risk even more than colonization with methicillin-susceptible *S. aureus* (MSSA), in both patients and healthy individuals [4-7]. In a North-American cohort of almost 30 000 patients who underwent MRSA screening at hospital admission, MRSA carriers had a 20-fold increased odds of developing MRSA bacteraemia compared with non-carriers [8]. In healthy athletes and soldiers, CO-MRSA colonization was associated with a notable increased risk for developing skin and soft tissue infections [4,9]. Decolonization therapy has been proven to reduce *S. aureus* infections in hospitalized patients, most pronounced in surgical patients [10-13]. Although evidence is limited, a 1-year survival benefit of *S. aureus* decolonization before clean surgical procedures is reported [14], as well as cost-effectiveness of active surveillance and decolonization at hospital admission [15].

However, data on the effectiveness of individual decolonization strategies in CO-MRSA carriage are scarce. This review discusses the evidence concerning strategies for elimination of MRSA colonization, with particular emphasis on CO-MRSA.

Methods

We searched PubMed from inception to 31 July 2023, using a combination of keywords to capture MRSA, colonization, and decolonization (search strategy in supplement). In addition, we hand-searched key references and international guidelines to identify citations not captured in the PubMed search. Screening was performed by one reviewer, and in case of uncertainty, a second reviewer was consulted. We screened 1335 titles and abstracts, and 129 articles were selected for a comprehensive full-text review. Studies published in languages other than English were excluded in the full-text review phase. Finally, 66 studies were included in this review. All studies were compiled in EndNote.

Results

Determining eligibility for eradication treatment

An important but complex question remains, which MRSA carriers should undergo eradication treatment. Worldwide differences in policies and attitudes towards MRSA carriage in the community exist between non-endemic and endemic areas. In countries with high MRSA prevalence, e.g. the United States, eradication treatment is not routinely recommended [16]. Some countries with low MRSA prevalence, e.g. the Netherlands and Denmark, successfully implemented a nationwide 'search and destroy' policy in the 1980s, targeting MRSA colonization [17,18]. This policy consists of screening and pre-emptive isolation of patients with an increased risk of MRSA carriage when hospitalized and subsequent decolonization treatment when persistent carriage is found. Two years after eradication treatment, 87% of CO-MRSA carriers in a non-endemic setting remained MRSA negative [19].

A major limitation in the generalizability of a 'search and destroy' approach to regions with high MRSA prevalence in the community is the high risk of recolonization. Currently, in countries with endemic MRSA, short-term *S. aureus* load reduction is often pursued to reduce infection risk in intensive care unit and surgical patients, either universally or targeted at MRSA carriers (or both MRSA and MSSA carriers) after screening [20]. This temporary suppression of MRSA is efficient in presurgical circumstances [21], but to prevent CO-MRSA transmission, complete eradication is desirable.

At an individual level, risk factors for failure of decolonization therapy can be a reason to refrain from pursuing this goal. Known risk factors for failure are indwelling catheters or medical devices, skin lesions, colonization of household contacts, chronic pulmonary disease, and an immunocompromised status [22,23].

As a result, two main factors should guide the decision for eradication therapy in an individual patient. First, the treatment goal, which can be either long-term eradication to prevent community transmission and infections, or short-term load reduction to prevent nosocomial infections and transmission. Second, the likelihood of long-term success of decolonization treatment, influenced by both the presence of individual risk factors for failure and the prevalence of MRSA in the environment, driving the risk of recolonization (Figure 1).

Lastly, when considering eradication treatment, potential adverse effects should be weighed in. This includes well-known effects such as (hepato-)toxicity and risk of *Clostridioides difficile* infection, but also newer insights such as potential disruption of the human microbiome [24].

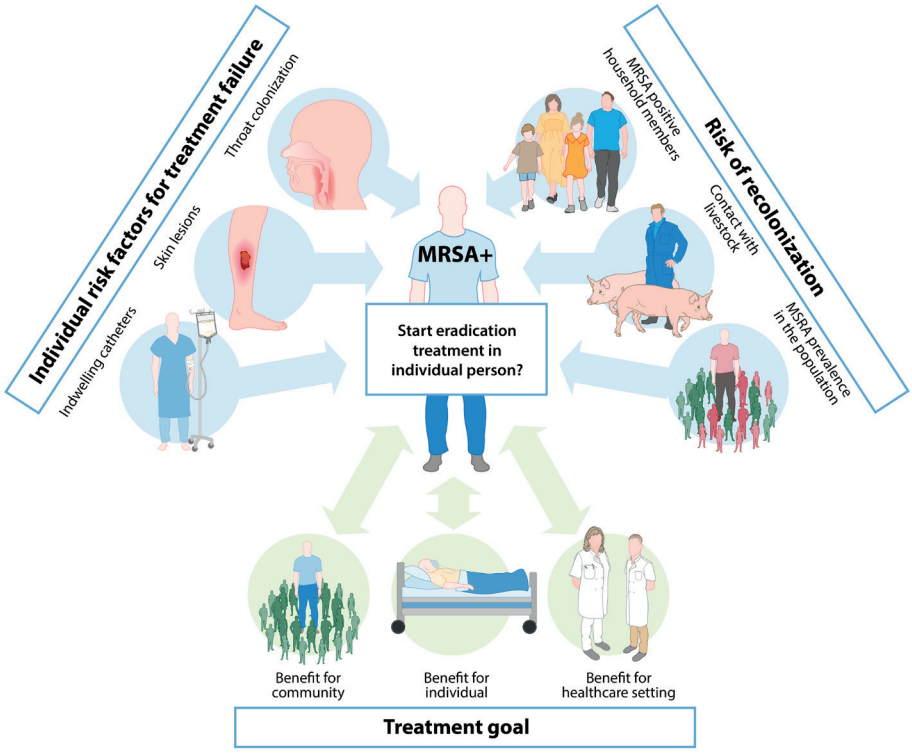

Figure 1. Factors of influence on the decision for eradiation treatment in an individual MRSA carrier. The decision to start eradication therapy in an MRSA carrier should be guided by the treatment goal and the likelihood of long-term success of decolonization treatment, influenced by both the presence of individual risk factors for failure and the prevalence of MRSA in the environment, driving the risk of recolonization. MRSA, methicillin-resistant *Staphylococcus aureus*.

Strategies for eradication therapy

MRSA eradication therapy usually exists of either topical - i.e. nasal ointment and skin wash-therapy alone or a combination of topical and systemic anti-staphylococcal agents. Topical therapy is proven to be effective in nasal-only carriage and in temporary (presurgical) load reduction [25,26]. In contrast, in patients with other body sites positive for MRSA, eradication with mupirocin and chlorhexidine skin wash is reported to be insufficient [27-29]. In a randomized controlled trial (RCT) of hospitalized patients colonized with MRSA on multiple body sites in a hospital

with endemic MRSA, mupirocin was only marginally effective [26]. In particular, throat carriage is associated with failure of topical eradication treatment [30]. In a small study on Swedish outpatients with MRSA throat carriage, topical therapy led to successful eradication in only 13%, as compared with 61% when topical therapy was combined with systematic antibiotics [31]. Positive household contacts were simultaneously treated. A similar outcome was reported in outpatient MRSA carriers in Canada initially; however, after 1 year, success rates with and without systemic antibiotics were found to be equal [32]. Canada is a high-endemic area, and because no screening of household contacts or genotyping was performed, it remains undetermined whether this outcome resulted from recolonization with a different strain, or long-term failure of eradication treatment.

Discriminating between nasal-only and extra-nasal MRSA colonization to guide optimal eradication therapy seems appropriate considering the abovementioned studies and from a pathophysiologic perspective. This distinction is also made in the Dutch MRSA eradication guideline, where mupirocin-sensitive, nasal-only MRSA carriage with intact skin is considered 'uncomplicated' and is recommended to be treated with topical agents only. MRSA carriers with extra-nasal colonization or other risk factors for (topical) treatment failure, e.g. active skin lesions and foreign body material, are considered 'complicated' and are treated with additional systemic antimicrobial agents [33]. This specific approach led to sustained decolonization in 85% of carriers after 1 year of follow-up [23].

MRSA carriage of household members was the most frequently encountered risk factor for CO-MRSA infections in Denmark between 1999 and 2006 [34], and was associated with failure of eradication treatment [22]. This emphasizes the need for screening and simultaneous eradication of all positive household members, especially in case of treatment failure.

In general, infection prevention and control measures are crucial in preventing further spread of MRSA [35], but are not included in this review.

Efficacy of topical decolonization therapy

The most commonly used topical treatment for *S. aureus* decolonization is mupirocin nasal ointment, which achieves its antimicrobial effect by inhibiting bacterial protein synthesis. It is often combined with daily antiseptic body wash. Mupirocin nasal ointment was proven to be effective in MSSA decolonization in the 1980s and 1990s [36-45]. In a systematic review that included studies analysing both MSSA and MRSA colonization, mupirocin resulted in negative MRSA cultures in 94% of patients after 1 week [25]. This percentage decreased to 65% after (mid- to long-term) follow-up.

All RCTs on topical MRSA eradication treatment are summarized in Table 1 [26,27,29,46-53].

Very high MRSA decolonization success rates have been reported with mupirocin treatment in a prospective study in hospitalized patients (98%), and an RCT involving long-term care facility residents (93%) [53,54]. Furthermore, in a retrospective analysis of MRSA-colonized patients who were readmitted during the study period, mupirocin was associated with being MRSA negative at readmission, compared with no treatment [55,56].

Focusing specifically on MRSA eradication in the community, little evidence is available on the effectiveness of mupirocin [57]. In an RCT involving 134 healthy MRSA-colonized American soldiers, mupirocin led to 88% nasal eradication compared with 65% with placebo after 8 weeks of follow-up [51]. Similarly, in 87 German hospital workers with nasal MRSA colonization, who were withdrawn from work until MRSA free, treatment with mupirocin nasal ointment and antiseptic mouth rinse and body wash resulted in successful eradication in 84% at 3 months of follow-up [58]. Prolonged mupirocin decolonization treatment (twice monthly for 5 days during 6 months) after discharge in patients that had been hospitalized in the United States with MRSA infections led to a higher decolonization rate compared with placebo (OR of colonization ¼ 0.44) [46].

Conflicting results on the effectiveness of mupirocin in CO-MRSA have been reported in regions with high MRSA prevalence, which may be indicative of an increased risk of recolonization rather than treatment failure. In an RCT comparing topical with systemic treatment in patients treated at a dedicated MRSA outpatient clinic, initial decolonization was achieved in 13 of 25 patients who received topical treatment, but this decreased to three after 12 months [32]. The vast majority of patients in this study were colonized at multiple body sites. Seven days of mupirocin nasal ointment combined with antiseptic body wash compared with placebo did not improve decolonization rate in 49 outpatients living with HIV in a RCT [50]. In addition, in a study involving 223 households with ambulatory MRSA skin and soft tissue infections, persistent MRSA colonization was similar in households with and without topical decolonization after 6 months of follow-up [49].

A concern with the use of mupirocin is the emergence of mupirocin resistance [59]. The prevalence of mupirocin resistance varies widely and is reported to be associated with its increased use [60]. Remarkably, a *post-hoc* analysis of the REDUCE-MRSA trial showed an overall low prevalence of mupirocin-resistant isolates and no increase after mupirocin decolonization treatment [61].

Table 1. Randomized trials on topical MRSA decolonization treatment

Author, year	Country	N	Population	Treatments	Duration[a]	Follow up[a]	Culture site(s)[b]	Decolonized	Other outcome
Miller, 2023 [51]	US	2121	Inpatients, post-discharge	1. Education 2. + mupirocin + chx	2x/month 5 days for 6 months	270	N,T,A, G,W	1. 57% 2. 73% (p <0.01)	
Pooveli-kunnel, 2018 [57]	Ireland	100	14% outpatients 86% inpatients	1. Medical-grade honey + Tricolsan 2. Mupirocin + Tricolsan	5	short-term	N, G, W	1. 43% 2. 57% (p 0.20)	Received 2 treatment courses: 1. 78% 2. 20%
Landelle, 2016 [59]	Switzer-land	146	Inpatients	1. Polyhexanide 2. Placebo	10	28	N, G	1. 34% 2. 29% (p 0.56)	
Cluzet, 2016 [53]	US	149	Households with SSTI	1. Education 2. + mupirocin + chx 3. + mupirocin + chx + reminders	7	180	N,A,G	1. +-80% 2. +-80% 3. +-80%	Time to clearance: 1. 19 days 2+3. 23 days
Weintrob, 2015 [52]	US	49	Outpatients with HIV	1. Mupirocin + chx 2. Placebo	7	180	N,A,G, T,P	1. 67% 2. 67%	
Fritz, 2011 [28]	US	300	Patients with SSTI + MSSA/MRSA colonization	1. Education 2. + mupirocin 3. + mupirocin + chx 4. + mupirocin + bleach baths	5	120	N,A,G	1. 48% 2. 56% (p 0.40) 3. 54% (p 0.51) 4. 71% (p 0.02)	Nasal decolonization: 1. 50% 2. 77% (p <0.01) 3. 76% (p <0.01) 4. 85% (p <0.01)
Ellis, 2007 [49]	US	134	Healthy soldiers	1. Mupirocin 2. Placebo	5	56	N	1. 88% 2. 65%	
Wendt, 2007 [26]	Germany	114	In- and outpatients, nursing home residents	1. Mupirocin + chx 2. Mupirocin + placebo	5	30	N,T,G, P	1. 8% (p 0.47) 2. 13%	Groin decolonization: 1. 93% (p <0.01) 2. 82%
Dryden, 2004 [58]	UK	224	Inpatients	1. Mupirocin + chx 2. Tea tree oil	5	14	N,T,G, S,W	1. 49% 2. 42% (p 0.03)	Nasal decolonization: 1. 86% 2. 58% (p <0.01)
Mody, 2003 [45]	US	127	Long term care facility residents with MRSA/ MSSA colonization	1. Mupirocin 2. Placebo	14	30	N,W	1. 88% (p <0.01) 2. 13%	
Harbarth, 1999 [25]	Switzer-land	98	Inpatients	1. Mupirocin + chx 2. Placebo + chx	5	26	N,G,U, W	1. 25% (p 0.40) 2. 18%	Nasal decolonization: 1. 44% 2. 23%

Legend: Chx, chlorhexidine; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; SSTI, skin and soft tissue infection. [a] In days. [b] N = nasal, A = axilla, G = groin, T = throat, P = perineum/rectum, W = wounds/skin lesions, S = sputum, U = urine.

Given the risk of the emergence of mupirocin resistance, alternative topical therapies have been evaluated. Medical-grade honey was only marginally inferior to mupirocin in decolonizing nasal MRSA colonization in a small RCT [47]. Topical therapy with tea tree preparations was significantly less effective compared with mupirocin-based topical therapy for the clearance of intranasal MRSA colonization [52]. Polyhexanide was not effective in MRSA decolonization compared with placebo in an RCT [48], and inferior to mupirocin and chlorhexidine in a retrospective analysis [62].

Efficacy of decolonization therapy with addition of systemic antibiotics

Using systemic antibiotics in addition to the topical treatment for MRSA decolonization is common practice in case of extra-nasal colonization in some countries, reserved for cases of topical treatment failure in others, and seldom or never employed in a third category of countries. Most studies on systemic treatment for MRSA decolonization have been performed in health care settings, with a high heterogeneity of treatment agents and control groups. All RCTs on systemic MRSA eradication treatment are summarized in Table 2 [31,32,63-68].

The combination treatment consisting of antiseptic body wash, intranasal mupirocin, rifampin, and trimethoprim/sulfamethoxazole or doxycycline was highly effective in MRSA decolonization of hospitalized patients [63,69,70]. In a small RCT in long-term care facilities in the United States, rifampin monotherapy was superior to no treatment, as well as to minocycline monotherapy. Combination therapy with rifampin and minocycline was not superior to rifampin alone. The majority of patients had decubitus and indwelling catheters, and after 3 months only half of the treated patients remained MRSA negative [66]. Moreover, the risk of emerging resistance when using rifampin monotherapy makes this an inappropriate approach. Another randomized trial on oral fusidic acid monotherapy or no treatment showed no difference in MRSA decolonization rate in 16 intensive care unit patients. However, the study was terminated because of emergence of fusidic acid-resistant strains [64]. Two cohort studies on trimethoprim/ sulfamethoxazole plus rifampin in hospitalized patients resulted in 64e66% successful MRSA decolonization [71,72]. Oral vancomycin, combined with topical therapy, was effective in eradicating MRSA-colonized staff and residents of a nursing home during an outbreak, although 80% experienced side effects [73].

Compared with topical therapy with mupirocin only, the combination of oral trimethoprim/sulfamethoxazole plus topical fusidic acid (without mupirocin) performed marginally worse in MRSA eradication in hospitalized patients and personnel after 14 days [65]. Rifampin plus novobiocin resulted in a non-significant higher decolonization rate after 14 days compared with rifampin plus trimethoprim-sulfamethoxazole (respectively 67% vs. 53%) in an RCT on MRSA-colonized patients and personnel in the United States. Decolonization in both groups was significantly more often achieved in colonization sites other than wounds [67]. However, novobiocin has since been withdrawn from the market. Rifampin with ciprofloxacin was more effective compared with rifampin with trimethoprim/sulfamethoxazole in an RCT on MRSA-colonized patients (50% vs. 37% eradicated after 6 months of follow-up). Only 21 patients were enrolled when the study was terminated because of emergence of ciprofloxacin resistance in the hospital, unrelated to the study [68].

Few studies have been published specifically on systemic MRSA decolonization in the community, mainly from countries with low MRSA prevalence. The previously mentioned Swedish study randomly assigned 52 outpatients with MRSA throat carriage between chlorhexidine, nasal mupirocin, rifampin, and either clindamycin or trimethoprim/sulfamethoxazole (group 1) and chlorhexidine and nasal mupirocin only (group 2). At 6 months of follow-up, 61% of systemically treated vs. 13% of topical treated patients were successfully decolonized ($p < 0.01$) [31]. In a cohort of Dutch outpatients with extra-nasal MRSA colonization, decolonization treatment combination of chlorhexidine body wash, mupirocin ointment intranasally, and a combination of two systemic antibiotics (mostly rifampin with trimethoprim or doxycycline) was successful in 85% of patients and the vast majority was still negative after 1 year of follow-up [23]. Two Danish cohort studies did not show a benefit of adding clindamycin to decolonization treatment of MRSA throat carriage [74,75].

In the previously discussed Canadian study, a country with high MRSA prevalence, 98 outpatients with MRSA colonization at any site were randomized between a 7-day course of topical treatment alone or supplemented with oral rifampin and doxycycline [32]. The initial success rate was higher in the systemic treatment arm, but this difference had disappeared after 12 months of follow-up. As said, no genotyping was performed to elucidate whether this was because of long-term treatment failure or recolonization with a different strain.

Table 2. Randomized trials on systemic MRSA decolonization treatment

Author, year	Country	N	Population	Treatments	Duration[1]	Follow-up[1]	Culture site(s)*	Decolonized at end of follow-up
Eum, 2021 [31]	Canada	98	Outpatients and inpatients	1. Mupirocin + chx 2. Mupirocin + chx + rifampin + doxycycline	7	365	N,P,W	1. 32% 2. 50% (p 0.04)
Lindgren, 2018 [30]	Sweden	52	Outpatients with throat colonization	1. Mupirocin + chx + rifampin + clindamycin/ SXT 2. Mupirocin + chx	7	180	N,T,P,W	1. 61% 2. 13% (p <0.01)
Simor, 2007 [62]	Canada	146	Inpatients	1. Mupirocin + chx + rifampin + doxycycline 2. No treatment	7	90	N,P,W,D	1. 74% 2. 32% (p <0.01)
Chang, 2000 [65]	Taiwan	16	ICU patients	1. Fusidic acid 2. No treatment	7	28	N,T,W,S	1. 33% 2. 50% (p 0.95)
Parras, 1995 [69]	Spain	84	13% HCW and 87% inpatients	1. Mupirocin + chx 2. SXT + topical fusidic acid + chx	5	28	N	1. 96% 2. 95% (p >0.05)
Muder, 1994 [64]	US	35	Long term care facility residents	1. Rifampin 2. Minocycline 3. Rifampin + minocycline 4. No treatment	5	90	N,U,W	1. 67% 2. 38% 3. 50% 4. 14%
Walsh, 1993 [70]	US	94	HCWs and inpatients	1. Novobiocin + rifampin 2. SXT + rifampin	7	14	N,G,W, S	1. 67% 2. 53% (p 0.18)
Peterson, 1990 [71]	US	21	Inpatients	1. Ciprofloxacin + rifampin 2. SXT + rifampin	14	180	N,G,W	1. 27% 2. 40% (p >0.1)

Legend. [1] In days. [2] N = nasal, A = axilla, G = groin, T = throat, P = perineum/rectum, W = wounds/skin lesions, S = sputum, U = urine, D = medical device or catheter exit site. Chx = chlorhexidine. SXT = trimethoprim/sulfamethoxazole. ICU = intensive care unit. HCW = healthcare worker

Future perspectives

Concerns about emerging resistance and the impact on the microbiome resulting from current treatment strategies drive the search for alternative, non-antibiotic, decolonization therapies. A recently published phase-two trial showed promising results of oral probiotics for nasal and intestinal *S. aureus* decolonization, with a 95% reduction of *S. aureus* colonization without notable changes in the microbiota [76]. Ongoing research is focused on engineering a skin probiotic to selectively combat MRSA colonization [77]. In addition, novel non-antibiotic drugs are being evaluated for their potential in *S. aureus* eradication, including the porphyrin drug XF-73, the LL-37- derived peptide P10 and SAAP-148 [78-80], and bacteriophage therapy [77].

Despite multiple attempts, vaccines to prevent *S. aureus* infections have so far not been proven clinically effective [81]. However, the high burden of disease provides grounds to continue the search.

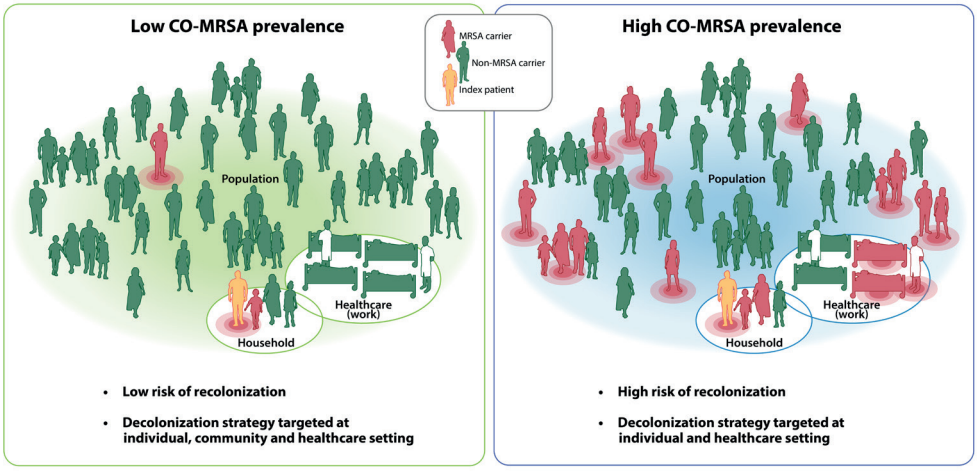

Figure 2. Implications of prevalence of MRSA carriage for the approach of the community and individual. In a low CO-MRSA prevalent setting, sustained decolonization of CO-MRSA is feasible and can prevent further spread in the community. This supports the 'search and destroy' policy, in which carriers are identified, household contacts screened, and decolonization is attempted. In this setting, this policy is effective in maintaining a low MRSA prevalence. In contrast, in high-endemic regions, there is high risk of recolonization. Consequently, routine eradication treatment of CO-MRSA aiming at achieving a non-carrier state for a prolonged period of time is less likely to be successful. In this setting, a standard 'search and destroy' policy is not likely to reduce the high MRSA prevalence, and an individualized approach is more rational. CO-MRSA, community-onset methicillin-resistant *Staphylococcus aureus*.

Discussion and conclusion

MRSA decolonization has been proven to reduce infections in both patients and healthy individuals. However, determining eligible treatment candidates and applying experiences and results from countries with low MRSA prevalence to countries with high MRSA prevalence continue to be challenging. In general, eradication studies in high prevalence areas are hampered by the indistinguishability of failing eradication treatment vs. recolonization. The likelihood of successful long-term decolonization is lower in a high endemicity setting compared with a low endemicity setting, because of the heightened risk of recolonization (Figure 2). Thus, both treatment goal (short-term bacterial load reduction in health care settings vs. long-term eradication in community settings), and likelihood of successful prolonged eradication should guide the eligibility for CO-MRSA decolonization treatment in the individual patient.

Although highly effective in decolonization of nasal MRSA carriage, the combination of mupirocin and antiseptic body wash appears to be insufficient in patients with extra-nasal MRSA colonization. The addition of systemic antibiotics is a rational approach in this patient category, but studies on systemic treatment of extra-nasal MRSA decolonization are subject to a high heterogeneity of treatment agents and comparator groups. Most evidence support a combination of topical therapy with rifampin and another antimicrobial agent for extra-nasal MRSA eradication. Future research would gain clinical applicability from reporting the carrier status of household contacts, long-term follow-up cultures, and reporting genotyping in case of failure. Eradication treatment with probiotics holds promise as a novel non-antibiotic strategy.

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Supplement 1. Seach strategy

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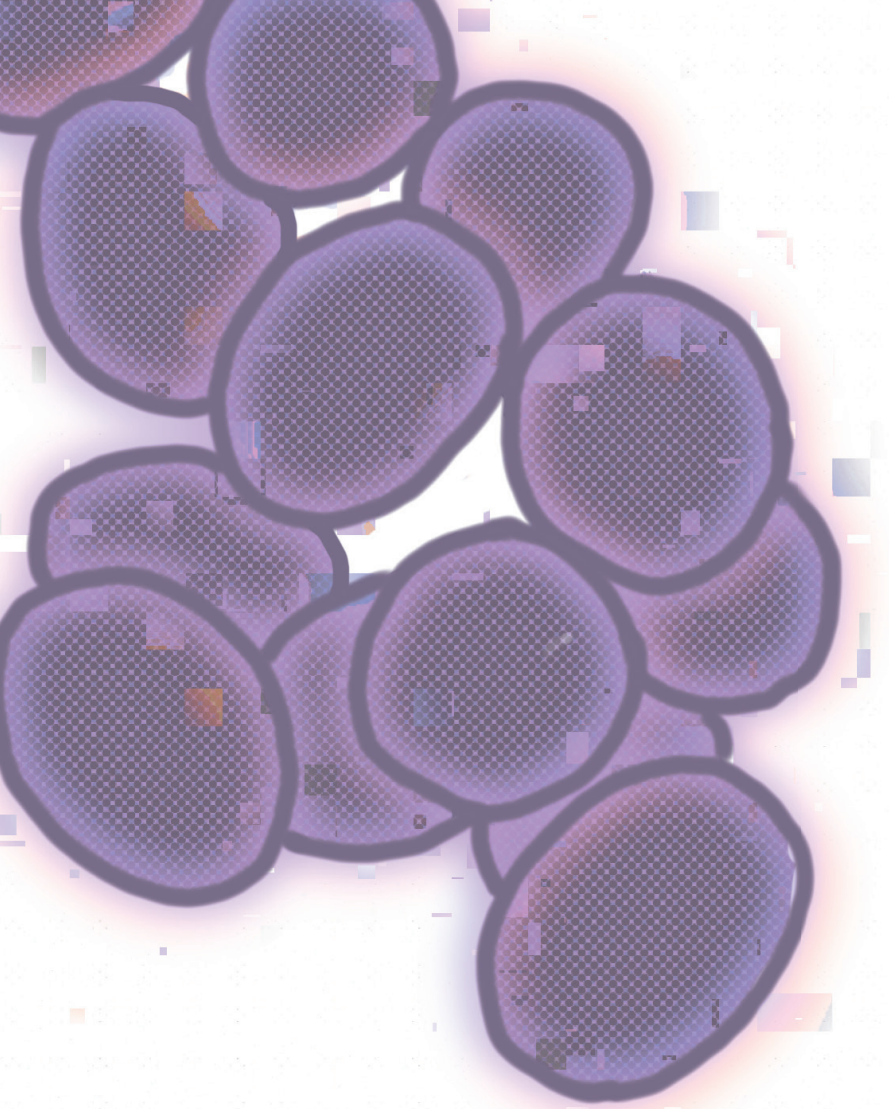
AND (“Carrier State”[majr] OR “colonization”[ti] OR “colonisation”[ti] OR “coloniz*”[ti] OR “colonis*”[ti] OR “carrier”[ti] OR “carriers”[ti] OR “carriage”[ti] OR “carriership*”[ti] OR “Nasal Cavity/microbiology”[majr]))) AND (“eradication*”[ti] OR “eradicat*”[ti] OR “treatment*”[ti] OR “decolonization*”[ti] OR “decolonisation*”[ti] OR “decoloniz*”[ti] OR “decolonis*”[ti] OR “elimination”[ti] OR “eliminat*”[ti]) NOT (“Animals”[mesh] NOT “Humans”[mesh]) AND (english[la] OR dutch[la])) OR ((“Staphylococcus aureus colon*”[ti] OR “s aureus colon*”[ti] OR “Staphylococcus aureus carr*”[ti] OR “s aureus carr*”[ti] OR ((“Staphylococcus aureus”[majr] OR “staphylococcus aureus”[ti] OR “s aureus”[ti]) AND (“Carrier State”[Mesh] OR “colonization”[tw] OR “colonisation”[tw] OR “coloniz*”[tw] OR “colonis*”[tw] OR “carrier”[tw] OR “carriers”[tw] OR “carriage”[tw] OR “carriership*”[tw] OR “Nasal Cavity/microbiology”[Mesh]))) AND (“eradication*”[tw] OR “eradicat*”[tw] OR “treatment*”[tw] OR “decolonization*”[tw] OR “decolonisation*”[tw] OR “decoloniz*”[tw] OR “decolonis*”[tw] OR “elimination”[tw] OR “eliminat*”[tw]) NOT (“Animals”[mesh] NOT “Humans”[mesh]) AND (english[la] OR dutch[la]) AND (systematic[sb] OR “meta-analysis”[pt] OR “meta analysis”[tw] OR “clinical trial”[pt] OR “clinical trial”[tiab] OR “clinical trials as topic”[mesh] OR “clinical trials”[tiab] OR “control groups”[mesh] OR “control group”[tiab] OR “control groups”[tiab] OR “controlled clinical trial”[pt] OR “controlled clinical trials as topic”[mesh] OR “cross-over studies”[mesh] OR “cross over study”[tiab] OR “cross over studies”[tiab] OR “double-blind method”[mesh] OR “double blind”[tiab] OR “evaluation studies as topic”[mesh] OR “follow-up studies”[mesh] OR “follow up study”[tiab] OR “follow up studies”[tiab] OR “placebos”[mesh] OR placebo*[tiab] OR placebos*[tiab] OR “pragmatic clinical trial”[pt] OR “prospective studies”[mesh] OR “prospective study”[tiab] OR “prospective studies”[tiab] OR “RaCT”[tiab] OR “RaCTs”[tiab] OR “random allocation”[mesh] OR “randomised “[tiab] OR “randomized controlled trial”[pt] OR “randomized controlled trials as topic”[mesh] OR “randomized”[tiab] OR random*[tiab] OR “RCT”[tiab] OR “RCTs”[tiab] OR “Research Design”[MeSH:noexp] OR “Research design”[tiab] OR “Research designs”[tiab] OR “single blind”[tiab] OR “single-blind method”[mesh] OR ((single*[tiab] OR double*[tiab] OR triple*[tiab]) AND (blind*[tiab] OR mask*[tiab])) OR volunteer*[tiab] OR “trial”[ti] OR “trials”[ti] OR “Multicenter Study”[Publication Type] OR “Cohort Studies”[Mesh] OR “Observational Study”[Publication Type])))



Chapter 4

Complicated carriage with methicillin-resistant *Staphylococcus aureus*: evaluation of the effectiveness of decolonization regimens advised in the Dutch national guideline

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Abstract

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization leads to increased infection rates and mortality. Decolonization treatment has proven to prevent infection and reduce transmission. As the optimal antimicrobial strategy is yet to be established, different regimens are currently prescribed to patients. This study aimed to evaluate efficacy of the decolonization treatments recommended by the Dutch guideline.

Methods

A retrospective multicenter cohort study was conducted in five Dutch hospitals. All patients who visited the outpatient clinic because of complicated MRSA carriage between 2014 - 2018 were included. We obtained data on patient characteristics, clinical and microbiological variables relevant for MRSA decolonization, environmental factors, decolonization regimen and treatment outcome. The primary outcome was defined as three negative MRSA cultures after treatment completion. Outcomes were stratified for the first-line treatment strategies.

Results

A total of 131/224 patients were treated with systemic antibiotic agents. Treatment was successful in 111/131 (85%) patients. The success rate was highest in patients treated with doxycycline-rifampicin (32/37, 86%), but the difference with any of the other regimens did not reach statistical significance. There was no difference in success rate of a 7-day treatment compared to 10-14 days of treatment (OR 0.99, 95%CI 0.39-2.53, p=1.00). Side effects were reported in 27/131 (21%) of patients and consisted mainly of mild gastrointestinal complaints. In a multivariable analysis, an immunocompromised status was an independent risk factor for failure at the first treatment attempt (OR 4.65, 95%CI 1.25-17.25, p=0.02).

Conclusion

The antimicrobial combinations recommended to treat complicated MRSA carriage yielded high success rates. Prolonged treatment did not affect treatment outcome. A randomized trial is needed to resolve whether the most successful regimen in this study (doxycycline plus rifampicin) is superior to other combinations.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a challenging global health problem. Colonization with MRSA leads to increased infection risks, ranging from mild skin infections to severe clinical syndromes, i.e. pneumonia and bloodstream infection [1-3]. Compared to infections with their more susceptible counterpart, mortality is high in MRSA infections. [4] This may in part be attributed to decreased antibiotic effectiveness and increased toxicity of the antibiotic therapy.
Decolonization of MRSA in carriers has proven to be an effective preventive strategy in reducing infection- and hospitalization rates [5, 6]. In Europe, the prevalence of MRSA in *Staphylococcus aureus* blood isolates was 16.4% in 2018 with large inter-country variations [7]. In the Netherlands, the MRSA prevalence in blood culture isolates is 1.4%, along with the Scandinavian countries one of the lowest in the world [7, 8]. The low prevalence in the Netherlands is to a large part ascribed to the 'search and destroy policy', targeting MRSA carriers [9-11]. The aim of this policy is to minimize colonization and transmission in both health care workers (HCWs) and patients. Active screening e.g. after hospitalization abroad, isolation of MRSA carriers and pre-emptive isolation of risk groups are part of this policy [11]. The policy also urges for decolonization treatment in all MRSA carriers.

The Dutch guideline for the treatment of MRSA carriage differentiates between complicated and uncomplicated carriership [12]. Uncomplicated carriership, i.e. exclusively located in the nose and without active infection, is advised to be treated with topical therapy (mupirocin topically applied to the nares) and hygienic measures. In case of complicated MRSA carriage additional systemic antimicrobial therapy with a combination of two antibiotic agents is recommended. Due to the limited availability of data [13-17], it has yet remained undecided which combination of anti-staphylococcal agents is most effective. The individual treatment regimen, i.e. the choice of antibiotic agents and treatment duration in clinical practice is therefore variable [18]. The aim of this study was to describe the effectiveness of different MRSA decolonization treatments for complicated MRSA carriage.

Table 1: Oral antibiotic combination therapy for decolonization of MRSA colonization according to the Dutch national guideline

	Antibiotic agent 1	**Antibiotic agent 2**
Recommended	Doxycycline 200mg qd **or** Trimethoprim 200mg bid	Rifampicin 600mg bid
Alternative	Clindamycin 600mg tid **or** Clarithromycin 500mg bid **or** Ciprofloxacin 750mg bid **or** Fusidic acid 500mg tid	Fusidic acid 500mg tid

Legend: qd = once a day, bid = twice a day, tid = three times a day.

Methods

A multicenter retrospective cohort study was conducted in five Dutch hospitals (one university hospital and four large regional teaching hospitals).

Study population

All consecutive patients referred to the outpatient clinic with complicated MRSA colonization from January 2014 until December 2018 were eligible for inclusion. Exclusion criteria were the absence of MRSA colonization upon screening at the outpatient clinic, uncomplicated carriership and a patient's objection to the use of his medical file for research purposes.

Outpatient clinic

History taking and physical examination were performed during the first visit to the outpatient clinic. Physical examination included skin examination, as skin lesions such as eczema may impede effective decolonization. Furthermore, physical examination involved examination of the oral cavity. Culture swabs were routinely obtained from nose, throat and perineum. If skin lesions e.g. wounds were present, additional cultures were obtained from these sites. Household contacts were screened as well, and colonized household contacts were treated simultaneously and were included in the study. The standard treatment consisted of nasal mupirocin thrice daily, topical disinfectants daily (chlorhexidine soap and betadine shampoo) and hygienic measures. Hygienic measures included daily change of underwear, clothes and towels as well as change of bed linen on day 1, 2 and 5. The first choice recommended systemic antibiotic agent combinations were doxycycline-rifampicin and trimethoprim-rifampicin, according to the in vitro susceptibility (12). Alternative combinations were either rifampicin or fusidic acid in combination with clindamycin, clarithromycin or ciprofloxacin, or rifampicin and fusidic acid (Table 1). Standard duration of antibiotic treatment was a minimum of 7 days.

Microbiological methods

Culturing and susceptibility determination was performed according to the Dutch Society of Medical Microbiology guideline for laboratory detection of highly resistant microorganisms. Minimum inhibitory concentration (MIC) breakpoints and zone diameter breakpoints for resistance and intermediate sensitivity were based on EUCAST criteria (19).

Data collection

The electronic patient files were reviewed to record patient characteristics, clinical data relevant for MRSA decolonization (e.g., immune status and skin diseases), environmental factors (e.g., health care profession, household members) and microbiological data (culture results and antimicrobial susceptibility patterns). In each hospital, the prescribed antibiotic therapy and treatment duration for all treatment episodes were extracted from the hospital electronic prescribing system. Microbiological data were retrieved from the Department of Medical Microbiology of each hospital.

Definitions

Uncomplicated MRSA carriership was defined as the presence of MRSA exclusively located in the nose and no active infection with MRSA and in vitro sensitivity for mupirocin and the absence of active skin lesions and the absence of foreign material that connects an internal body site with the outside (e.g., urine catheter, external fixation material) and no previously failure of decolonization treatment. All other situations were considered complicated colonization [12]. An 'isolated patient' was defined as a solitude carrier without any known family or household members with MRSA colonization. In case of any known positive family or household member, these patients together were considered a cluster. A household member was defined as a person sharing the same house by day and night and sharing a bedroom and/or bathroom, and/or living room and/or kitchen [12]. Immunocompromised status was defined as either a hematologic malignancy, stem cell transplantation, organ transplantation, immunosuppressive medication (e.g., chemotherapy, steroids) or HIV infection. The primary outcome of the study was success rate of decolonization treatment, defined by three times negative MRSA cultures from swabs taken from nose, throat and perineum. The first culture needed to be taken at least 48 hours after treatment, with the follow-up cultures obtained with one-week intervals. The long-term success rate was defined as an additional set of negative MRSA swabs one year after decolonization treatment (data available for four hospitals).

Statistical analysis and outcome

Data were presented as rates (percentages or proportions) for categorical variables and as medians plus interquartile range (IQR) for continuous variables. The overall success rate of decolonization treatment was presented as a rate, with 95% confidence interval (95%CI), and was stratified for different treatment strategies.

In univariate analysis, Odds ratio's (with 95% confidence intervals) and Fisher's exact tests were applied to identify clinical risk factors of treatment failure. In the multivariable regression analyses variables from univariate analysis with a $p<0.05$ were included, together with variables that were previously reported to be associated with treatment failure: MRSA throat carriage and perineal carriage [20, 21].

Ethical approval

Ethical approval was granted by the institutional ethical review committee of the Leiden University Medical Center and the participating hospitals.

Results

During the study period, 224 patients were referred to the outpatient departments because of MRSA colonization. Because of absence of colonization or uncomplicated carriership at the first evaluation, respectively 27 and 20 patients were excluded. Of the remaining 177 patients, only 131 received systemic antibiotics (Figure 1). Reasons for not starting decolonization with systemic antibiotics were spontaneous clearance of colonization (14/177; 8%), lost to follow up (6/177; 3%) and/or acceptance of colonization (23/177; 13%). Reasons for accepting colonization were either related to a high risk of failure, i.e. therapy resistant skin lesions in eczema, or to a high risk of recurrence, i.e. frequent livestock contact or regular visits to health care facilities abroad. Three patients (3/177; 2%) were successfully treated with topical therapy only.

The patient characteristics of all 177 patients with complicated colonization and of the 131 patients with complicated colonization that were treated with systemic antibiotic therapy are summarized in Table 2. Of the 131 patients with complicated colonization and treatment with systemic antibiotics, 19 (15%) lived alone, 103 (79%) lived with one or more household members and in 9 patients (7%) data on household members were missing. In 91/103 (88%) patients all household members were screened for carriership. In 5/103 (5%) only part of the household members were screened and in 7/103 (7%) none of the household members were screened. In total, 229 household members were screened, of which 91 (40%) tested positive for MRSA.

Decolonization treatment

In 131 patients systemic antibiotic treatment was prescribed (Figure 1), and in 125/131 (95%) the choice of antibiotic regimen was in line with the national guideline (Table 1). Six patients received antimicrobial combinations that were not in line with the guideline and 4 others were initially treated with hygienic measures and topical therapy only. The success rate of the first decolonization attempt was 97/131 (74%). Not all patients that failed on a first treatment were treated again. Of the 34 patients in whom the first decolonization attempt failed, 17/34 (50%) underwent a second treatment (Table 3). The success rate after this second treatment was 11/17 (65%). Of the remaining six patients, four were treated for a third time, which was successful in 3/4 (75%) of patients. The cumulative success rate was 111/131 (85%). Mean follow-up time was 13 months. In 78/111 (70%) of the initially successfully treated patients follow-up cultures at T≥12 months were available. In 4/78 (5%) of patients these cultures were positive for MRSA. Side effects were reported in 27/131 (21%) of patients and consisted of gastrointestinal complaints (21/131; 16%) and malaise (4/131; 3%). An allergic reaction occurred in 1 of the 131 patients.

Figure 1 (next page). Flowchart of treatment schedule. Uncomplicated MRSA carriership was defined as the presence of all of the following features: (i) MRSA exclusively located in the nose, (ii) no active infection with MRSA, (iii) in vitro sensitivity for mupirocin, (iv) the absence of active skin lesions, (v) the absence of foreign material that connects an internal body site with the outside (e.g., urine catheter, external fixation material), and (vi) no previous failure of decolonization treatment. All other cases were considered complicated. Successful decolonization was defined by three successive negative MRSA swabs from nose, throat, and perineum at least 48 h after treatment, with a minimum interval of 1 week. An asterisk (*) indicates that colonization was accepted under certain circumstances, e.g., active noncurable skin lesions, short life expectancy, wishes of the patient, or a high risk of recurrence due to frequent livestock contact or regular visits to health care facilities abroad. An arrowhead indicates patients added to another group.

Figure 1. Flow chart

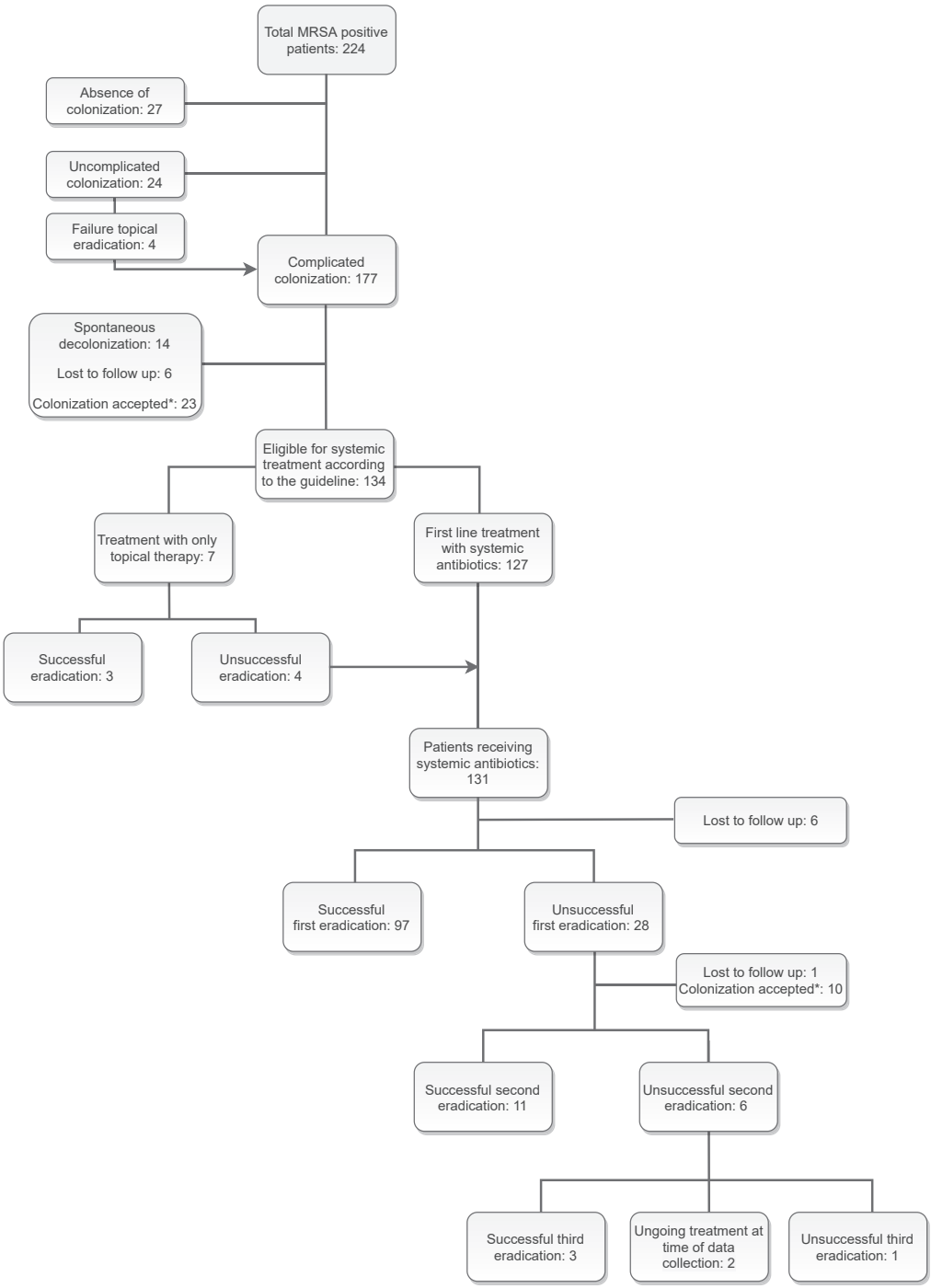

Table 2: Patient characteristics

	All patients with complicated MRSA colonization	**Patients receiving treatment with systemic antibiotics**
	N = 177 (100%)	**N = 131 (100%)**
Male sex	82 (46)	64 (49)
Age, median (IQR)	41 (12-70)	43 (13-73)
Positive household member)	76 (43)	61 (47)
Risk factors for colonization		
Immunocompromised status	17 (10)	12 (9)
Chronic antibiotic use	7 (4)	7 (5)
Health care worker	27 (15)	22 (17)
Professional livestock contact	4 (2)	3 (2)
Reason for MRSA screening prior to referral		
Positive household member	44 (25)	29 (22)
Contact with positive person in health care facility	32 (18)	26 (20)
Infection with MRSA	59 (33)	42 (32)
Screening after contact livestock	2 (1)	0
Screening after foreign hospital	25 (14)	22 (17)
Other	8 (5)	7 (5)
Unknown	7 (4)	4 (3)
Site of colonization		
Nose	118 (67)	88 (67)
Throat	114 (64)	87 (66)
Perineum	98 (55)	70 (53)
Other (e.g. skin lesions, infection sites)	58 (33)	40 (31)
Reason for complicated colonization		
Extranasal colonization	166 (94)	122 (93)
Foreign material internal-external	6 (3)	2 (2)
Mupirocin resistance	4 (2)	4 (3)
Skin lesions	33 (19)	24 (18)
Previous unsuccessful decolonization	20 (11)	14 (11)
Infection during colonization		
MRSA infection*	65 (37)	45 (34)

Table 2 continued

		All patients with complicated MRSA colonization	**Patients receiving treatment with systemic antibiotics**
		N = 177 (100%)	**N = 131 (100%)**
Microbiology results			
PVL	Present	36 (20)	27 (21)
	Absent	78 (44)	61 (47)
	n/a	63 (36)	43 (32)
Rifampicin	Susceptible	158 (89)	119 (91)
	Resistant	4 (2)	4 (3)
	n/a	15 (9)	8 (6)
Trimethoprim/sulfamethoxazole	Susceptible	136 (77)	103 (79)
	Resistant	27 (15)	20 (15)
	n/a	14 (8)	8 (6)
Clindamycin	Susceptible	111 (63)	79 (60)
	Resistant	43 (24)	36 (28)
	n/a	23 (13)	16 (12)
Doxycycline	Susceptible	72 (41)	60 (46)
	Resistant	38 (22)	28 (21)
	n/a	67 (37)	43 (33)

Legend: The first column includes all 177 patients with complicated colonization. The second column depicts the 131 (out of these 177) patients that received treatment with systemic antibiotics. Values are count (%) for categorical variables and median (IQR= interquartile range) for continuous variables. PVL = Panton Valentine Leucocidin. *MRSA infection = culture confirmed infection(s) with MRSA during colonization.

Table 3: Follow-up after decolonization treatment

		Follow-up cultures after treatment ***Total treated patients = 131***		
		Week 1	Week 2	Week 3
First decolonization attempt	Available	130	111	103
	Positive	14	8	6
Second decolonization attempt	Available	17	15	13
	Positive	2	2	2
Third decolonization attempt	Available	4	4	3
	Positive	0	1	0

Legend: Follow-up cultures after decolonization treatment. Values are count. After one positive culture, no further follow-up cultures were performed.

Table 4: Decolonization success rates of antibiotic regimens

Antibiotic agents	**Treated first attempt**	**Successful after first attempt**	**Treated second attempt**	**Successful after second attempt**	**Treated third attempt**	**Successful after third attempt**
Doxycycline + rifampicin	37	32 (86%; 71-96)	1	1	0	-
Trimethoprim* + rifampicin	60	41 (68%; 55-80)	8	5	3	2
Clindamycin + rifampicin	19	15 (79%; 54-94)	2	1	1	1
Other	15	9 (60%; 32-84)	6	4	0	-
Total	131	97 (74%)	17	11 (65%)	4	3 (75%)

Legend: Values are count (%; 95% confidence interval). The most frequently used combinations of antibiotic agents are mentioned, the 8 other antibiotic regimens are bundled in 'other'. *Trimethoprim was with or without sulfamethoxazole. 'First attempt' is the first attempt with systemic antibiotic agents added to the treatment, i.e. first treatment episode in complicated colonization or second treatment episode after failure of first treatment with topical treatment in uncomplicated colonization.

Antibiotic regimens

For the treatment of complicated colonization in this cohort, 12 different combinations of antibiotic agents were prescribed with a duration ranging from 5 to 14 days. The most frequently prescribed combinations of antibiotic agents were doxycycline-rifampicin, trimethoprim (with or without sulfamethoxazole)-rifampicin and clindamycin-rifampicin. The success rates of the different antibiotic combinations at the consecutive decolonization attempts are summarized in Table 4. In the first treatment attempt, the combination of doxycycline-rifampicin showed the highest success rate (32/37, 86%) compared to trimethoprim(/sulfamethoxazole)-rifampicin (41/60, 68%), clindamycin-rifampicin (15/19, 79%) and 'other regimens' (9/15, 60%). The difference in success rate at first attempt of doxycycline-rifampicin versus all other regimens did not reach statistical significance (86 versus 69%, OR 2.20, 95%CI 0.77-6.31, p=0.16). There was no difference in outcome of addition of trimethoprim alone (success rate 19/24, 79%; 95%CI 58-93) or in combination with sulfamethoxazole (success rate 22/31, 71%; 95%CI 52-86).

Prolonged antibiotic treatment (10-14 days) was not associated with better treatment outcome (49/64; 77%) compared to a 7-day treatment (40/51; 78%) (OR 0.99, 95%CI 0.39-2.53, p=1.00). There was a trend towards a higher success rate in the patients in whom the guideline for treatment choice was followed (88/115; 77%) compared to the patients in whom the guideline was not followed (6/12; 50%, 95%CI 0.97-10.94, p=0.08).

Predictive variables

In the univariate risk analysis, being part of a known household cluster (OR 2.38, 95%CI 1.01-5.61, p= 0.05) and an immunocompromised status (OR 6.27, 95%CI 1.81-21.68, p <0.01) were associated with failure at first decolonization attempt (Table 5).

Panton Valentin Leucocidin (PVL) was tested in 88 patients and was positive in 27/88 (31%). There was no correlation between PVL positivity and success of eradication in these patients (OR 0.57, 95%CI 0.15-1.82, p=0.36).

In the multivariable analysis an immunocompromised status remained an independent risk factor for failure at the first treatment attempt (OR 4.83, 95%CI 1.34-17.45, p=0.02) (Table 5).

Table 5: Univariate and multivariable analysis of predictive variables for failure of first decolonization attempt

	Univariate analysis		Multivariable analysis		
Variable	**OR (95%CI)**	**p-value**	**B**	**OR (95%CI)**	**p-value**
Patient characteristics					
Age >60y	0.68 (0.23-1.98)	0.61			
Male sex	1.54 (0.66-3.58)	0.39			
Part of a known household cluster	2.38 (1.01-5.61)	0.05	0.60	1.83 (0.74-4.51)	0.19
Healthcare worker	0.54 (0.15-1.99)	0.56			
Comorbidities					
Immunocompromised status	6.27 (1.81-21.68)	<0.01	1.58	4.83 (1.34-17.45)	0.02
Current skin disease	0.66 (0.21-2.11)	0.59			
Chronic antibiotic use	1.83 (0.32-10.53)	0.61			
MRSA infection*	1.29 (0.54-3.08)	0.65			
Site of colonization other than nose~					
Throat culture positive	0.84 (0.34-2.11)	0.81	0.07	1.07 (0.39-2.96)	0.89
Perineum culture positive	1.51 (0.62-3.71)	0.39	0.40	1.49 (0.57-3.90)	0.42
Other site culture positive	1.20 (0.49-2.97)	0.81			
PVL genes					
PVL positive	1.56 (0.49-4.93)	0.54			

Legend: Results of univariate and multivariable analyses. Values are OR=odds ratio (95%CI= 95% confidence interval), B= regression coefficients. PVL= Panton Valentine Leucocidin. *MRSA infection = culture confirmed infection(s) with MRSA during colonization. ~ = Sites of colonization reflects positive cultures at screening. Multiple sites could be positive within one patient.

Discussion

The main finding of our study is the success rate of decolonization of 74% after the first treatment attempt, which is relatively high when compared to previous literature. In the Dutch study by Ammerlaan et al. in 2011, this rate was 56% [18]. A possible explanation for this difference may be that the guideline adherence for treatment choice was much lower in the study by Ammerlaan (62%) compared to our study (90%). A second explanation may be that in our study – in the majority of cases – household members were screened and treated simultaneously, preventing failure because of recolonization by untreated colonized household contacts. In the time of the study by Ammerlaan et al, according to the Dutch guideline, household members were only screened if the first decolonization attempt had failed. Routine screening of household members before starting treatment was not included in the guideline until 2012.
The success rate of topical treatment in combination with systemic antibiotics – in our study – is decidedly high compared to topical treatment without systemic antibiotics in the literature, supporting the current guideline. Earlier studies have shown a success rate of approximately 40% after the first decolonization attempt in patients that were treated with topical treatment alone [21, 22].

There were no apparent differences in success rates between different antibiotic regimens. The combination of doxycycline-rifampicin had the highest success rate but this did not reach statistically significance. This combination is one of the first choice regimens in the Dutch guideline. There was no difference in effectivity between a treatment duration of 7 days as compared to 10-14 days. This supports the guideline recommendation of a minimum antibiotic treatment of 7 days [12].

Being part of a known household cluster and immunocompromised status were associated with failure at the first treatment attempt. In multivariable analysis only immunocompromised status remained an independent risk factor for failure at the first treatment attempt, although there were few patients (12) in this group. This differs from an earlier study by Ammerlaan et al, in which chronic pulmonary disease, ADL dependency, throat carriage, perineal carriage and the presence of a device were associated with treatment failure [20]. This difference may be explained by the difference in study population, as Ammerlaan et al did not exclude uncomplicated carriers from their analyses.

The fact that 27/224 (12%) of the referred patients were no longer colonized with MRSA at the time of visiting the outpatient clinic is a relevant observation. It illustrates the possibility of spontaneous clearance and the importance of repeated screening before starting treatment.

In the current search and destroy strategy, MRSA carriers are exposed to systemic antibiotic therapy, for the benefit of society, even if they are asymptomatic. The side-effects of treatment should be weighed against the benefits of a search and destroy policy. Reported side effects in this study were mild and the effectivity of decolonization high, supporting the current that MRSA decolonization strategy in a low prevalence country like the Netherlands.

There are several limitations of our study. Due to its observational design, confounding limits the determination of the most effective antibiotic strategy. However, so far there is only one small randomized trial published comparing the efficacy of ciprofloxacin-rifampicin and trimethoprim-sulfamethoxazole combinations in MRSA decolonization. This study showed no significant difference in success rates, but did not include a doxycycline based regimen and was underpowered [14]. The majority of previously published studies are limited to the comparison of different antibiotic combinations versus topical treatment alone or no treatment at all [15, 17]. A second limitation of our study is that group sizes are small due to the low prevalence of MRSA colonization and the variety of different antibiotic regimens that were prescribed, reflecting the current guideline. A third limitation is that a proportion of patients were lost to follow-up one year after treatment. However, only 5% of the initially successfully treated patients that were cultured after one year were recolonized with MRSA. In the study of Lekkerkerk et al. [23], the median number of days to detect a MRSA recurrence was 24 and 12% of recurrences was detected between 62 and 200 days. Therefore, the majority of recurrences is expected to have been detected in our study, but late recurrences may have been missed. However, these late recurrences could also be ascribed to re-colonization from an unidentified source rather than to failure of the initial decolonization treatment.

In conclusion, treatment for complicated MRSA colonization according to the guideline has a high success rate. These findings endorse the current strategy of ‘search and destroy’. For future research, a randomized trial would be necessary to further distinguish whether doxycycline-rifampicin has a higher efficacy rate compared to alternative treatment combinations, as suggested in this study.

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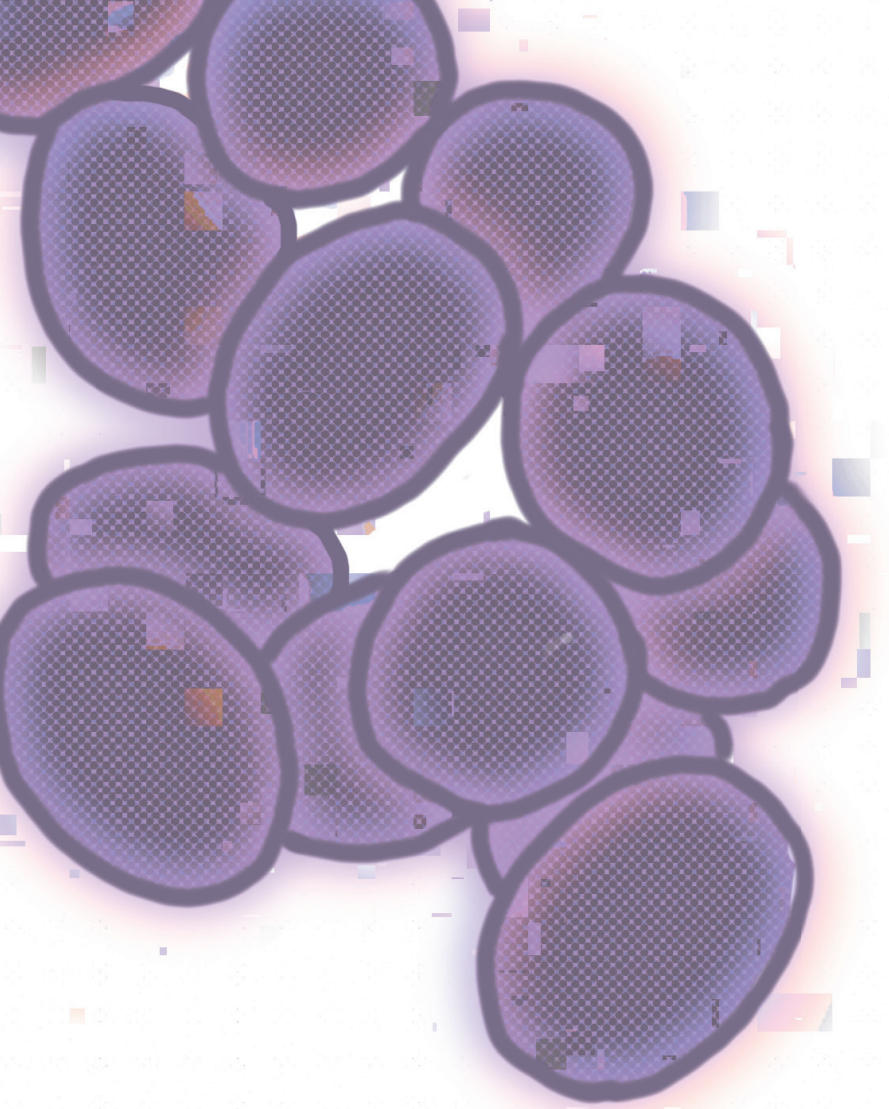

Chapter 5

Genetic determinants in MRSA carriage and their association with decolonization outcome

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization increases the risk of infection. Response to decolonization treatment is highly variable and determinants for successful decolonization or failure of eradication treatment are largely unknown. Insight into genetic predictors of eradication failure is potentially useful in clinical practice. The aim of this study was to explore genetic characteristics that are associated with MRSA decolonization failure. This cohort study was performed in a tertiary care hospital in the Netherlands. Patients with ≥ 1 positive MRSA culture from any site and with available whole -genome sequencing data of the MRSA isolate between 2017 and 2022 were included. Lineages, resistance, and virulence factors were stratified by MRSA decolonization outcome. In total, 56 patients were included: 12/56 (21%) with treatment failure and 44/56 (79%) with successful decolonization (with or without preceding treatment). A significant association was found between ciprofloxacin-resistant lineages and failure of eradication (OR 4.20, 95%CI 1.11–15.96, P=0.04). Furthermore, livestock-associated MRSA and the major community-associated MRSA lineages ST6-t304 and ST8-t008 were associated with successful eradication treatment or spontaneous clearance. In conclusion, this explorative study showed a higher eradication failure rate in complicated MRSA carriers with ciprofloxacin-resistant MRSA lineages, which are predominantly healthcare-associated. Further studies are warranted to confirm the higher eradication failure risk of ciprofloxacin-resistant lineages, and identify the underlying mechanisms.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health threat with high morbidity and mortality rates [1]. Colonization with MRSA leads to increased infection rates of up to 25% [2, 3]. The Netherlands has one of the lowest levels of endemic MRSA in the world [4]. This low prevalence is for a large part attributed to a successful 'search and destroy' policy aiming at MRSA carriage, that has been executed for over three decades [5]. This policy consists of screening and pre-emptive strict isolation of patients with increased risk of MRSA carriage when hospitalized and subsequent decolonization treatment when carriage is found. Response to decolonization treatment is highly variable; in some patients, eradication treatment fails despite multiple attempts, in others colonization is self-limiting without treatment [6, 7]. Spontaneous clearance or persistent carriership is driven by a complex host–pathogen interaction, which is largely unraveled. Furthermore, antimicrobial treatment (i.e., eradication therapy) adds to this complex interaction, and introduces pharmacodynamic and pharmacokinetic effects. In summary, patient

characteristics, antibiotic regimen, and isolate characteristics are all considered to contribute to decolonization treatment outcomes [7–9].

Different MRSA clones have emerged throughout the world with a high variety in virulence factors [10]. The rapid developments in the field of genetic diagnostics, especially whole-genome sequencing (WGS), have expanded the knowledge of the complexity and heterogeneity of this pathogen. MRSA strains produce a broad range of virulence factors, such as toxins, immune evasion factors, and adhesion proteins [11]. These virulence determinants are mostly carried on mobile genetic elements (MGEs), such as pathogenicity islands, plasmids, or bacteriophages [3]. Furthermore, virulence determinants can vary between hospital-associated, community-associated, and livestock-associated (LA) MRSA strains [12].

WGS of MRSA strains has been deployed extensively for infection control purposes. It has proven to be of great value in the epidemiology and outbreak management of MRSA [13]. In addition, WGS allows for molecular characterization of isolates by identifying clinically relevant genetic determinants that can help to predict response to decolonization treatment. So far, microbial genomics is not yet broadly applied to identify determinants related to MRSA eradication treatment outcome [14]. As an example, the presence of Panton-Valentine leucocidin (PVL) genes and genes associated with mupirocin resistance were associated with successful eradication outcome [9, 15]. A recent study elaborated on genetic factors and carriage duration, and showed a potential role of bacteriophage-related chemotaxis inhibitory protein encoded by *chp* [8]. Insight into genetic predictors of eradication failure is potentially useful in clinical practice. Ultimately, differentiating between MRSA carriers that will benefit from an eradication treatment and carriers more prone to eradication failure may enable personalized medicine.

In this explorative pilot cohort study, we evaluated genomic characteristics that are associated with MRSA decolonization failure. This was established by linking WGS data of MRSA isolates to clinical patient characteristics.

Methods

This cohort study was conducted at the University Medical Center Groningen, a tertiary hospital in the Northern part of the Netherlands, between 2017 and 2022. The prevalence of MRSA carriage in the Netherlands during this time was < 1%. During these years, genetic analyses of first MRSA isolates (both from carriage and infection) had been performed in all index patients and most of the healthcare workers, for the purpose of surveillance and outbreak management. Genetic analysis was not performed in healthcare workers who were positive at their pre-employment

screening nor in positive family contacts of index patients. All patients (both adults and children) and healthcare workers of whom WGS of an MRSA isolate was performed were retrospectively identified and were screened to meet the selection criteria. Healthcare workers will be also addressed as 'patients' from now on in this manuscript, since they were treated as patients for this matter. Inclusion criteria were ≥ 1 visit to the outpatient infectious diseases clinic because of MRSA carriage or infection, ≥ 1 positive MRSA culture from any site, and available WGS data of the MRSA isolate. Exclusion criterion was the absence of follow-up cultures. Only the first available MRSA isolate per patient was included in the analysis. The patients had been assessed by the outpatient clinicians using protocols based on the national MRSA eradication guideline [16]. This includes in case of an MRSA infection, adequately treating the infection first, and subsequently screen for persistent colonization.

Data collection

Clinical data were extracted from the electronic patient files. This included demographics, complicated versus uncomplicated carriage, treatment regimen, duration of therapy, and follow-up cultures. MRSA culture results were extracted from the laboratory information system. This included initial and follow-up MRSA cultures, including minimal inhibitory concentrations (MICs) of antibiotics, phenotypic susceptibility results, and WGS results.

Microbiological methods

Culturing using BHI broth with 2.5% saline and MRSAid chromagar (bioMérieux, Lyon, France), susceptibility determination by automated susceptibility testing by VITEK2 (bioMérieux, Lyon, France), and cefoxitin disk diffusion were performed according to the Dutch Society of Medical Microbiology guideline for laboratory detection of highly resistant microorganisms as part of routine diagnostic procedures [17]. MIC breakpoints and zone diameter breakpoints for resistance and intermediate sensitivity were based on EUCAST criteria [18]. The isolates were identified as *S. aureus* by matrix-assisted laser desorption/ionization–time of flight mass spectrometry (Bruker Daltonics, Billerica, US). First MRSA isolates per patient were genotypically confirmed by Xpert MRSA NxG based on the detection of the *mecA* or *mecC* targets (Cepheid, Sunnyvale, US).

A total DNA extraction for whole-genome sequencing was performed directly from colonies of the respective isolates using the Ultraclean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, US) according to the manufacturer's protocol. DNA concentrations were determined using a Qubit® 2.0 fluorometer and the dsDNA HS and/or BR assay kit (Life Technologies, Carlsbad, CA, US). Subsequently, DNA libraries

were prepared using the Nextera XT v2 kit (Illumina, San Diego, CA, US) according to the manufacturer's instructions. Short-read sequencing was performed with an Illumina MiSeq System generating paired-end reads of 250 bp. De novo assembly of paired-end reads was performed using CLC Genomics Workbench v12.0.1-v20.0.4 (QIAGEN, Hilden, Germany) after quality trimming (Qs≥20) establishing a word size of 29.

Based on next generation sequencing data (ENA project number PRJEB59407), molecular typing was performed using Ridom Seqsphere+v8.3.1 (Ridom, Münster, Germany). Herewith multilocus sequence typing (MLST) ST type was derived and core genome multilocus sequence typing (cgMLST) was performed using a scheme including 1861 alleles [19]. Isolates with a maximum of 24 allelic differences were denominated the same complex type. Antibiotic resistance genes were identified by Resfinder v4.1 (Center for Genomic Epidemiology, Lingby, Denmark). A predefined set of virulence factors were identified using AlereMicroarray schemes in Ridom Seqsphere+v8.3.1 (Ridom, Münster, Germany) [20].

Definitions

Uncomplicated MRSA carriage was defined as having all of the following features: (i) the presence of MRSA exclusively located in the nose, (ii) no active infection with MRSA, (iii) in vitro susceptibility for mupirocin, (iv) the absence of active skin lesions, (v) the absence of foreign material that connects an internal body site with the outside (e.g., urine catheter, external fixation material), and (vi) no previously failure of decolonization treatment. All other carriage cases were considered complicated colonization. Uncomplicated carriage is advised to be treated with topical therapy (mupirocin topically applied to the nares, disinfecting shampoo) and hygienic measures. In cases of complicated MRSA carriage, additional systemic antimicrobial therapy with a combination of two antibiotic agents is recommended, according to the national guideline [16]. MRSA infection was defined as a positive culture send to the microbiology laboratory from an infected body site as indicated by the treating physician.

Successful decolonization was defined as three consecutive negative MRSA cultures from swabs taken from nose, throat, and perineum, with the cultures obtained at 1-week intervals, without antibiotic usage [16]. For analyses, patients were divided in two groups: patients with failure of eradication treatment (failure group) and patients with successful decolonization with or without preceding treatment (successful decolonization group).

Livestock-associated MRSA was defined based on the Spa-type. The Spa-types *t011, t034, t108, t567, t571, t588, t753, t779, t898, t899, t943, t1184, t1197, t1254, t1255, t1451, t1456, t1457, t2123, t2287, t2329, t2330, t2383, t2582, t2748, t2971, t2974, t3013, t3014, t3053, t3146*, and *t3208* were considered to be associated with livestock

[12]. All other Spa-types were considered to be not associated with livestock.

Statistical analysis

Data are presented as percentages or proportions for categorical variables and as medians plus interquartile range (IQR) for continuous variables. Univariate analysis was performed using Fisher's exact test. As this study has an explorative character, no adjustment for multiple testing was done.

Results

During the study period, 181 patients visited the MRSA outpatient clinic. WGS was performed in 56/181 (31%) patients and these were included in the study (Fig. 1). As shown in Figure 1, there were 12 patients with treatment failure (i.e., one in the uncomplicated carriage group and eleven in the complicated carriage group). All other patients (44) were MRSA negative at the end of follow-up and were defined as successfully decolonized (three in the uncomplicated carriage group, eight with MRSA infection without subsequent carriage, ten with spontaneous decolonization and 23 with successful treatment of complicated carriage). Patient and treatment characteristics of these two groups are depicted in Table 1. In the failure group, one patient out of twelve (8%) had uncomplicated carriage and 11/12 (92%) patients had complicated carriage. The successful decolonization group existed of 33/44 (75%) patients with complicated carriage, 3/44 (7%) patients with uncomplicated carriage, and 8/44 (18%) patients with MRSA infection, without subsequent carriage. Twenty-six out of 44 (59%) patients successfully underwent eradication treatment, in 10/44 (23%) patients colonization resolved spontaneously and 8/44 (18%) were treated for an MRSA infection, without subsequent eradication treatment. Of all 34 patients who underwent eradication treatment for complicated MRSA carriage, 11/34 (32%) had treatment failure. No significant differences in treatment characteristics were found between patients with treatment success and treatment failure (Table 1).

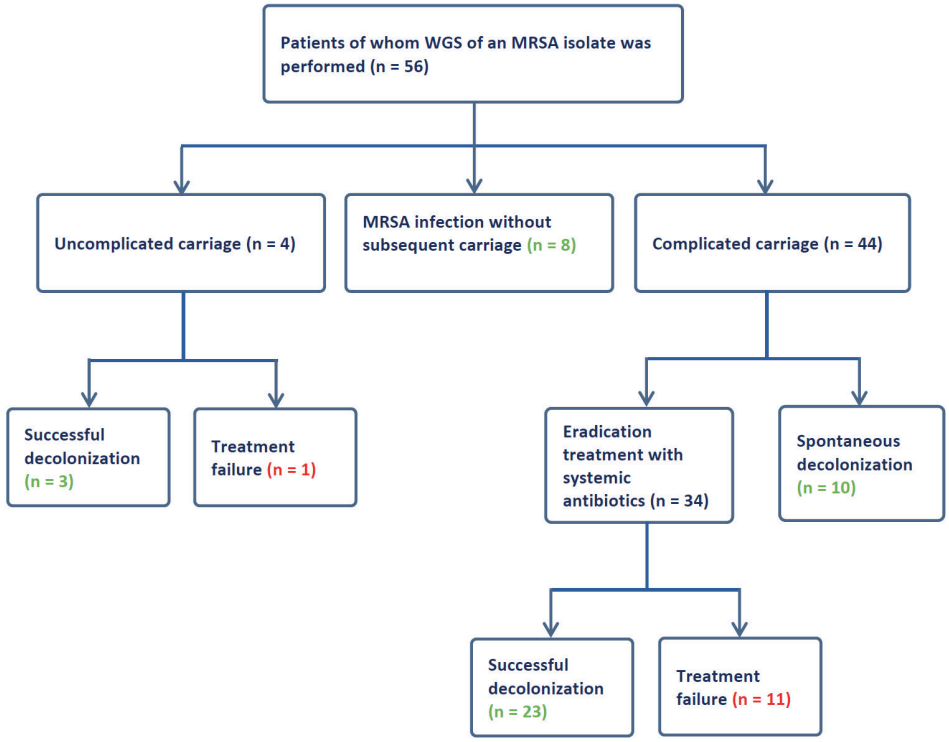


Figure 1. Flow chart. Flow chart of inclusions. MRSA carriage was defined as complicated in 44/56 (79%) patients, of whom 34/44 (77%) received systemic antibiotics as eradication treatment. The other 10/44 (23%) patients with complicated carriage were spontaneously cleared of MRSA before start of the planned eradication treatment. In addition, 8/56 (14%) patients with MRSA infections did not have subsequent MRSA carriage, and 4/56 (7%) patients had uncomplicated carriage. The green numbers represent the successful decolonization group (with or without preceding treatment). The red numbers represent the treatment failure group

Table 1

Baseline and treatment characteristics

Characteristic	Treatment failure *n*=12	Successful decolonization *n*=44	*P*
Sex, male (*n* (%))	6 (50.0)	25 (56.8)	0.75
Age (median (IQR))	23.5 (23)	35.5 (41)	0.11
Complicated carriage	11 (91.7)	33 (75.0)	1.00
Uncomplicated carriage	1 (8.3)	3 (6.8)	1.00
MRSA infection, no subsequent carriage	0	8 (18.2)	n.a
MRSA infection (*n* (%))	3 (25.0)	18 (41.9)	0.34
Treatment regimen (*n* (%))*			0.24
Rifampicin + doxycycline	3/11 (27.3)	9/23 (39.1)	
Rifampicin + cotrimoxazole	4/11 (36.4)	7/23 (30.4)	
Rifampicin + trimethoprim	4/11 (36.4)	3/23 (13.0)	
Rifampicin + clindamycin	0	3/23 (13.0)	
Vancomycin + clindamycin	0	1/23 (4.3)	
Treatment duration (*n* (%))*			
7-day treatment	10/11 (90.9)	19/23 (82.6)	1.00
14-day treatment	1/11 (9.1)	4/23 (17.4)	1.00

*This percentage represents the percentage of the patients who were treated with systemic antibiotics. Patients with uncomplicated carriage, MRSA infection without subsequent carriage, or spontaneous decolonization were not treated with systemic antibiotics

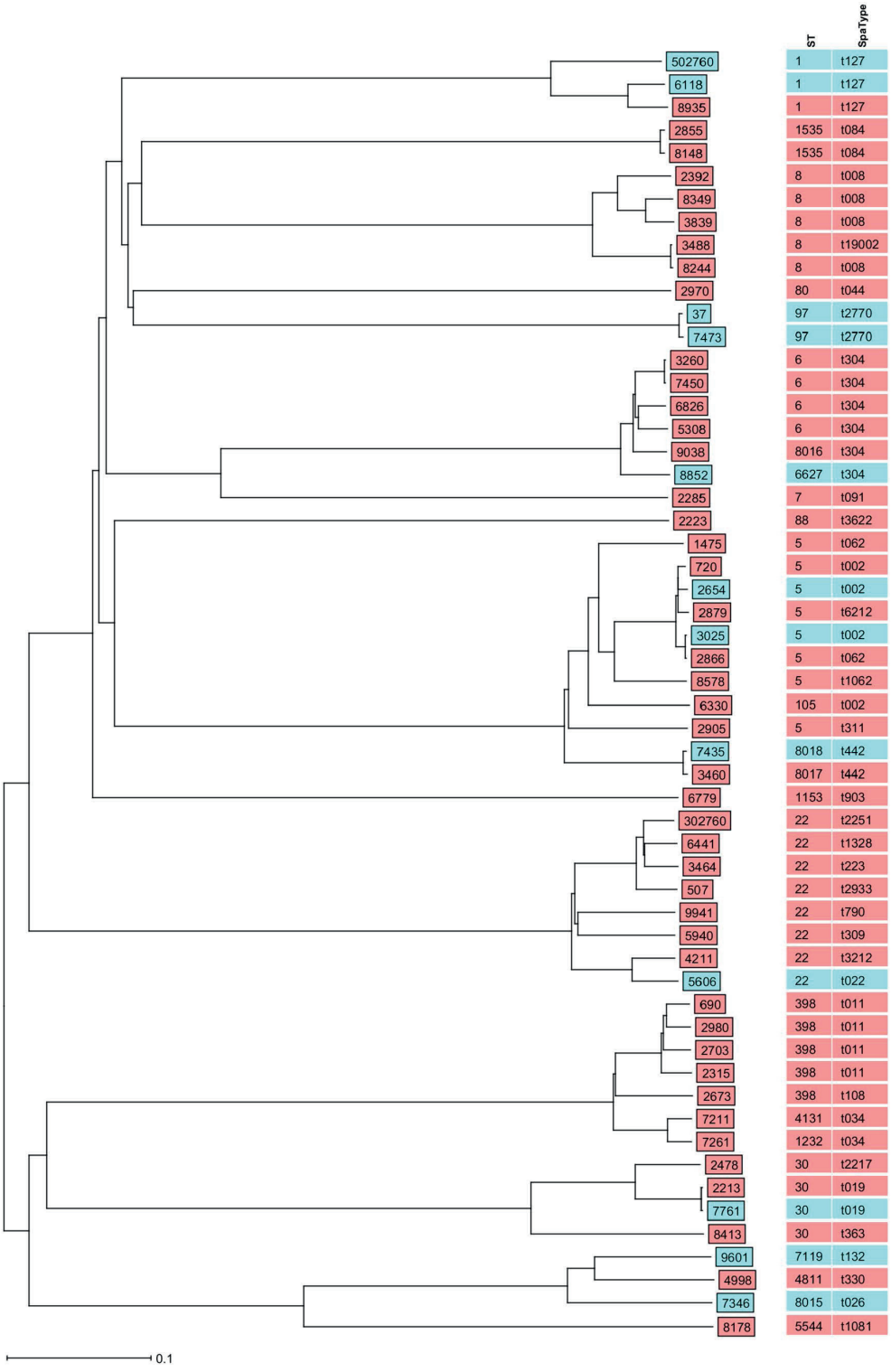
n.a. not applicable

Lineages

Among the 56 MRSA isolates, 24 different MLST types were represented. The most predominant MLST types were ST5 (8/56) and ST22 (8/56), followed by ST8 (5/56) and ST398 (5/56) (Figure 2 and Table S1). The complex types were mostly unique, only seven complex types were represented twice (2615, 4940, 6749, 9359, 10,282, 17,413, 24,737). All isolates (*n*=7) with livestock-associated Spa-types belonged to clonal complex 398. The non-livestock-associated MLST types ST1 (2/3), ST97 t2770 (2/2), ST6627 (1/1), and ST7119 (1/1) were more frequently or exclusively found in the failure group. In contrast, isolates of patients with successful decolonization predominantly belonged to community-associated lineages ST6-t304 (4/4), ST8-t008 (5/5), and the livestock-associated clonal cluster 398 (7/7) (Figure 2).

Figure 2 (next page). Phylogenetic tree of MRSA isolates.

Neighbor-joining tree from SeqSphere software based on curated schema where comparison of 1861 core genes of *S. aureus* was used. The study isolates from patients who failed on eradication treatment are presented in blue, and from patients with successful decolonization in pink. The corresponding isolate antibiotic susceptibility profiles are shown in supplementary table S1


ST
SpaType
502760 1 t127
6118 1 t127
8935 1 t127
2855 1535 t084
8148 1535 t084
2392 8 t008
8349 8 t008
3839 8 t008
3488 8 t19002
8244 8 t008
2970 80 t044
37 97 t2770
7473 97 t2770
3260 6 t304
7450 6 t304
6826 6 t304
5308 6 t304
9038 8016 t304
8852 6627 t304
2285 7 t091
2223 88 t3622
1475 5 t062
720 5 t002
2654 5 t002
2879 5 t6212
3025 5 t002
2866 5 t062
8578 5 t1062
6330 105 t002
2905 5 t311
7435 8018 t442
3460 8017 t442
6779 1153 t903
302760 22 t2251
6441 22 t1328
3464 22 t223
507 22 t2933
9941 22 t790
5940 22 t309
4211 22 t3212
5606 22 t022
690 398 t011
2980 398 t011
2703 398 t011
2315 398 t011
2673 398 t108
7211 4131 t034
7261 1232 t034
2478 30 t2217
2213 30 t019
7761 30 t019
8413 30 t363
9601 7119 t132
4998 4811 t330
7346 8015 t026
8178 5544 t1081
0.1

Susceptibility and resistance genes

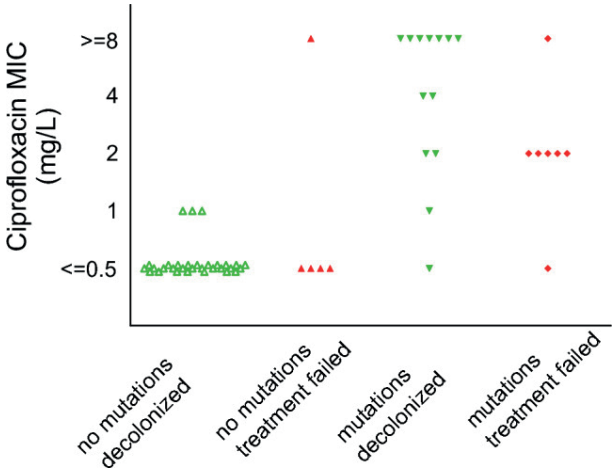
All MRSA isolates tested susceptible for the antibiotics used in the eradication treatments, and this was in line with the sequencing data that showed the absence of acquired resistance genes to these drugs (Table S2). Treatment failure was therefore not the result of resistance against the antibiotics used for the treatment. A significant association was found between ciprofloxacin resistance and failure of eradication (OR 4.20, 95%CI 1.11–15.96, $P=0.04$) (Table 2). None of the patients had been treated with ciprofloxacin. The ciprofloxacin-resistant isolates belonged to ST5 (5), ST8 (2), ST22 (3), ST30 (1), ST97 (2), ST105 (1), ST398 (1), ST5544 (1), ST7119 (1), and ST8018 (1). In the ciprofloxacin-resistant isolates ($n=18$), we detected one or more of the associated point-mutations S84L (10/18) in the gyrase GyrA, S80F (14/18) or S80Y (3/18) or E84G (2/18) or I45M (1/18) in the DNA topoisomerase IV GrlA, and P585S (1/18) in GrlB (Table S3). In the isolates of all patients with treatment failure, mutations associated with ciprofloxacin resistance were identified in 7/12 (58%) of the isolates, whereas in the isolates of patients with successful decolonization, these mutations were identified in 13/44 (30%) isolates (Figure 3). Two isolates with the unique point mutation I45M in GrlA did not show increased MICs to ciprofloxacin. All seven persons with ciprofloxacin-resistant MRSA with failure to eradication treatment were either healthcare workers, or most likely had acquired the MRSA during hospitalization or after medical interventions. Rifampicin resistance-associated point-mutations were found in four isolates (I527L [3/4] and D471Y [1/4] in rpoB). While all four of these isolates had a rifampicin MIC ≤ 0.03, these isolates belonged to four patients with treatment failure (Table S3). No other associations were found between phenotypic antibiotic resistance or resistance genes and failure of eradication treatment (Table 3).

Table 2. Phenotypic resistance to antibiotics used in eradication therapy

Antibiotic (R)	Treatment failure *N*=12 (%)	Successful decolonization *N*=44 (%)	*P*
Doxycycline	4 (33.3)	15 (34.1)	1.00
Ciprofloxacin	7 (58.3)	11 (25.0)	0.04
Trimethoprim	0 (0.0)	10 (25.6)	0.09
Cotrimoxazole	0 (0.0)	9 (20.9)	0.18
Clindamycin	6 (50.0)	15 (35.7)	0.50
Rifampicin	0	0	n.a
Mupirocin	0	0	n.a

Phenotypic resistance per antibiotic agent, stratified by decolonization outcome

Figure 3. Ciprofloxacin MIC according to MRSA decolonization outcome and mutations associated with ciprofloxacin resistance.



MIC range depicted by decolonization outcome and the presence of mutations (in GrlA, GrlB, or GyrA) associated with ciprofloxacin resistance. In the isolates of patients with treatment failure (red, n = 12), mutations were identified in 7/12 (58.3%) of the isolates, whereas in the isolates of patients with successful decolonization (green, n = 44), mutations were identified in 13/44 (29.5%) isolates. In isolates with high resistance (i.e., MIC > = 8mg/L) to ciprofloxacin (n = 9), multiple mutations were detected (Table S3), except for 1 isolate without mutations.

Table 3. Resistance genes

Genes	Treatment failure *N* = 12 (%)	Successful decolonization *N* = 44 (%)	*P*
erm(C)	4 (33.3)	7 (15.9)	0.22
erm(B)	0 (0)	1 (2.3)	1.00
erm(A)	2 (16.7)	7 (15.9)	1.00
tet(K)	4 (33.3)	13 (29.5)	1.00
tet(L)	1 (8.3)	0 (0)	0.21
tet(M)	0 (0)	5 (11.4)	0.57
tet(S/M)	0 (0)	5 (11.4)	0.57
dfrG	0 (0)	4 (9.1)	0.57
dfrK	0 (0)	1 (2.3)	1.00
fus(B)	0 (0)	1 (2.3)	1.00
fus(C)	2 (16.7)	4 (9.1)	0.60

Resistance genes stratified by decolonization outcome. No genes associated with mupirocin resistance were detected

Virulence factors

An overview of the distribution of virulence genes among the patients with eradication failure and patients with successful decolonization is presented in Table 4. No associations were found between virulence genes and failure of eradication. Remarkably, PVL (*lukF_PV* and *lukS_PV*) was found more often in patients with successful decolonization compared to the patients with eradication failure, although non-significant (30% vs 17%, $P=0.48$). The genes *lukF_PV* and *lukS_PV* and *spIE* were significantly associated with an MRSA infection ($P<0.05$). The genes *aur, hlgABC, icaACD, setB, setC, hlI, hlII, arcc, aroe, glpf, gmk, pta, tpi, yqil, isaB, lukX, lukY*, and *ebpS* were present in all isolates and were therefore excluded from the analysis. The genes *arc, edinABC, etABD, seb, sec*, and *sed* were only sporadically present and were therefore excluded from the analysis as well.

Discussion

In this study, we explored associations between MRSA isolate characteristics, genetic determinants, and decolonization outcomes in a Dutch population of MRSA carriers in a tertiary hospital. We found an association of eradication failure with carriage of ciprofloxacin-resistant healthcare-associated lineages, whereas livestock-associated MRSA lineage ST398 and the majority of community-associated MRSA lineages ST6-t304 and ST8-t008 were associated with successful eradication treatment or spontaneous clearance.

The failure rate in eradication treatment of complex MRSA carriers was higher compared to previous reports in Dutch studies [5, 7]. Our study was conducted in the outpatient clinic of a tertiary hospital, with consequently a more than average representation of healthcare workers or patients with an extensive history of hospitalizations. Such patients mainly carry healthcare-associated MRSAs, that are adapted to survive under harsh nosocomial conditions and antibiotic exposure.

In our study, we found an association between ciprofloxacin resistance and failure in eradication treatment. Remarkably, none of the patients had been treated with ciprofloxacin. The ciprofloxacin-resistant MRSAs in our study belonged to various lineages, including five isolates of the healthcare-associated ST5 lineage with single amino acid substitution in GrlA S80F. The mutation in this healthcare-associated lineage, and its association with fluoroquinolone resistance and the presence of virulence genes as enterotoxins, β-hemolysin converting phage, and leucocidins has been described previously [21].

Table 4. Virulence factors and genes stratified by decolonization outcome

Virulence factors	Genes	Treatment failure *N*=12 (%)	Successful decolonization *N*=44 (%)	*P*
Capsule type 5	*cap5H*	6 (50.0)	28 (63.6)	0.51
	cap5J	6 (50.0)	28 (63.6)	0.51
	cap5K	6 (50.0)	27 (61.4)	0.52
Capsule type 8	*cap8H*	5 (41.7)	16 (36.4)	0.75
	cap8I	6 (50.0)	16 (36.4)	0.51
	cap8J	6 (50.0)	16 (36.4)	0.51
	cap8K	6 (50.0)	16 (36.4)	0.51
Chemotaxis-inhibiting protein	*chp*	6 (50.0)	27 (61.4)	0.52
Enolase	*eno*	11 (91.7)	44 (100.0)	0.21
Fibrinogen-binding protein	*fib*	11 (91.7)	36 (81.8)	0.67
Leukocidin D/E	*lukD*	8 (66.7)	24 (54.5)	0.53
	lukE	8 (66.7)	23 (52.3)	0.52
Panton-Valentine leucocidin	*lukF_PV*	2 (16.7)	13 (29.5)	0.48
	lukS_PV	2 (16.7)	13 (29.5)	0.48
Staphylokinase	*sak*	11 (91.7)	34 (77.3)	0.67
Staphylococcal complement inhibitor	*scn*	11 (91.7)	38 (86.4)	1.00
Enterotoxin genes	*seg*	7 (58.3)	20 (45.5)	0.52
	sei	7 (58.3)	20 (45.5)	0.52
	sem	7 (58.3)	20 (45.5)	0.52
	sen	7 (58.3)	18 (40.9)	0.51
	seo	7 (58.3)	20 (45.5)	0.52
	seu	6 (50.0)	17 (38.6)	0.52
	seh	2 (16.7)	1 (2.3)	0.11
	sek	2 (16.7)	5 (11.4)	0.64
	seq	2 (16.7)	3 (6.8)	0.31
	sea_sep	2 (16.7)	9 (20.5)	1.00
	sej	1 (8.3)	6 (13.6)	1.00
	ser	1 (8.3)	6 (13.6)	1.00
Serine protease A/B/E	*splA*	8 (66.7)	20 (45.5)	0.33
	splB	8 (66.7)	21 (47.7)	0.33
	splE	5 (41.7)	13 (29.5)	0.50
Toxic shock syndrome toxin-1	*tst1*	0 (0.0)	6 (13.6)	0.32

The resistance to fluoroquinolones is generally high in healthcare-associated MRSA [22]. Successful hospital-adapted ciprofloxacin-resistant lineages have emerged among several nosocomial species as *E. coli*, *K. pneumoniae*, vancomycin-resistant *E. faecium*, and MRSA. These lineages have acquired stable point-mutations in gyrase and/or topoisomerase IV enzymes [23]. It is unsure what drives this evolution, besides the exposure to fluoroquinolones.

Both tolerance and persistence have been reported in low-level ciprofloxacin-resistant *E. coli*, allowing to survive exposure to therapeutic concentrations of ciprofloxacin [24]. In tolerance, bacterial cells survive using a "hibernation mode," in which the cell cycle and metabolism are temporarily stopped, preventing killing by antibiotics. In persistence, a bacterial subpopulation is able to survive antibiotic exposure [25]. Cross-tolerance to multi-drugs has been reported, but does not necessarily occur in all tolerant isolates and is dependent on antibiotic regimen and duration of exposure [26]. To the best of our knowledge, no studies have reported cross-tolerance in low-level ciprofloxacin-resistant *S. aureus* isolates to the antibiotic regimens in MRSA eradication used in this study. Therefore, the explanation for the association found in our study remains uncertain. Potentially, healthcare-associated MRSAs are more prone to failure of eradication treatment, and ciprofloxacin resistance may be a biomarker for these difficult-to-treat lineages.

The recent finding of association between *chp* and carriage duration was not found in our study [8]. Compared to the Danish study, our patient population had more healthcare-associated MRSA. Also, there is large heterogeneity in the Danish and Dutch MRSA treatment guidelines. The main difference is the more general use of two systemic antibiotics in the Netherlands, compared to sporadic systemic treatment in Denmark.

Two studies, in Denmark and Sweden, reported that PVL-positive isolates had a higher eradication success rate [15, 27]. We also found a higher (non-significant) rate of PVL-positive isolates in the successful eradication group, mainly belonging to the CA-MRSA linages ST30 and ST8-t008. However, associations do not necessarily reflect an etiologic cause, but can also reflect markers or confounders. We postulate that PVL is a marker of certain non-healthcare-associated MRSA lineages that are easier to eradicate, rather than a direct positive effect of the PVL toxin to eradication outcomes.

There are multiple factors of potential influence on MRSA eradication outcome. Carriers can reacquire MRSA isolates from contamination in their environment, or by positive household members. The eradication treatment of patients in this study was performed in a specialized outpatient clinic setting, following the Dutch eradication protocol [16]. Several measures are taken to prevent reacquisition, such as simultaneous treatment of positive household members and hygienic instructions. Isolate characteristics may also play a role in the risk of spread and reacquisition of MRSA. Hetem et al. showed that in a hospital setting, the transmission of livestock-associated MRSA was 4.4 times lower compared to non-livestock-associated MRSA isolates [12]. In general, MRSA isolates can be able to survive antibiotic exposure, despite having a MIC indicating susceptibility to the antibiotic agent. Our study showed that the antibiotic treatment failure is not explained by the common

acquired resistance genes related to resistance, of which the presence or absence corresponded to the phenotypic susceptibility in all isolates. However, alternative survival mechanisms to antibiotic exposure, such as tolerance and persistence, are not detectable by measuring MICs. Other potential factors influencing MRSA eradication outcome, e.g., therapy incompliance and host genetics [28], were not assessed in our study.

There are some limitations of this study. It is a single-center study with a small sample size, a heterogeneous population, and a limited number of failed treatments. In addition, we did not always confirm that treatment failure was caused by the same clone, or acquisition of a different MRSA. However, given the very low prevalence of MRSA in the Netherlands, this would be highly unlikely. Furthermore, we did not correct for multiple testing. However, since it is an explorative study in a relatively undiscovered subject, we believe the results are still valid and useful in targeting future research. For this explorative purpose, we focused on pathogen factors and only added a limited number of host characteristics (i.e., sex, age, and complicated versus uncomplicated carriership). Other host factors—including host genetics—may influence the risk of treatment failure as well. Lastly, we investigated genes with a previously reported role in virulence. Future genome-wide association studies could perhaps identify signatures with novel genetic factors implicated in intracellular survival and biofilm formation that predict eradication failure. However, this requires a larger and preferably prospective data set.

In conclusion, this explorative study showed a higher eradication failure rate in complicated MRSA carriers with ciprofloxacin-resistant MRSA lineages, which are predominantly healthcare-associated. In contrast, carriers of livestock-associated MRSA and the major community-associated ST8 and ST6 lineages were generally successfully decolonized. Further studies are warranted to confirm the higher eradication failure risk of ciprofloxacin-resistant lineages, and identify the underlying mechanisms. The identification of lineages that are prone to eradication failure is of clinical relevance, since it could influence the initiation and monitoring of MRSA eradication therapy.

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Supplementary data

Table S1. Isolate characteristics

Isolate	MLST	Spa	MIC VA	MIC DX	MIC CI	Disc TR	MIC TR	MIC SXT
37	97	2770	≤ 0.5	≥ 16.0	2	24		≤ 10.0
507	22	2933	≤ 0.5	≤ 1.0	≤ 0.5	-	6	160
690	398	011	≤ 0.5	≥ 16.0	≤ 0.5	6		80
720	5	002	≤ 0.5	≤ 1.0	2	-		≤ 10.0
1475	5	062	1	≤ 1.0	≤ 0.5	6		≤ 10.0
2213	30	019	1	≥ 16.0	≤ 0.5	21		≤ 10.0
2223	88	3622	1	≤ 1.0	≤ 0.5	25		≤ 10.0
2285	7	091	1	≤ 1.0	≤ 0.5	20		≤ 10.0
2315	398	011	≤ 0.5	≥ 16.0	1	25		≤ 10.0
2392	8	008	≤ 0.5	≤ 1.0	≤ 0.5	21		≤ 10.0
2478	30	2217	≤ 0.5	≤ 1.0	1	17		≤ 10.0
2654	5	002	1	≤ 1.0	2	23		≤ 10.0
2673	398	108	≤ 0.5	≥ 16.0	≤ 0.5	-		≤ 10.0
2703	398	011	1	≥ 16.0	≥ 8.0	23		≤ 10.0
2855	1535	084	1	≥ 16.0	≤ 0.5	23		≤ 10.0
2866	5	002	≤ 0.5	≤ 1.0	1	22		≤ 10.0
2879	5	6212	1	≤ 1.0	2	23		≤ 10.0
2905	5	311	≤ 0.5	≤ 1.0	4	6		160
2970	80	044	≤ 0.5	≥ 16.0	≤ 0.5	23		≤ 10.0
2980	398	011	≤ 0.5	≥ 16.0	≤ 0.5	6		≥ 320.0
3025	5	002	1	≤ 1.0	2	22		≤ 10.0
3260	6	304	1	≤ 1.0	≤ 0.5	22		≤ 10.0
3460	8017	442	1	≤ 1.0	≤ 0.5	21		≤ 10.0
3464	22	223	≤ 0.5	≥ 16.0	≤ 0.5	-		160
3488	8	008	≤ 0.5	≤ 1.0	≤ 0.5	19		≤ 10.0
3839	8	008	1	≥ 16.0	≥ 8.0	19		≤ 10.0
4211	22	294	1	≤ 1.0	≥ 8.0	20		≤ 10.0
4998	4811	330	≤ 0.5	≤ 1.0	≤ 0.5	19		≤ 10.0
5308	6	304	1	≤ 1.0	≤ 0.5	21		≤ 10.0
5606	22	022	≤ 0.5	≤ 1.0	≥ 8.0	27		≤ 10.0
5940	22	309	≤ 0.5	≤ 1.0	≥ 8.0	6		80
6118	1	127	1	≥ 16.0	≤ 0.5	23		≤ 10.0

MIC RA	MIC CL	Res VA	Res DX	Res CI	Res TR	Res SXT	Res RA	Res CL
≤ 0.03	0.25	S	R	R	S	S	S	S
≤ 0.03	0.25	S	S	S	R*	R	S	S
≤ 0.03	≥ 4.0	S	R	S	R	R	S	R
≤ 0.03	0.25	S	S	R		S	S	R
≤ 0.03	0.25	S	S	S	R	S	S	S
≤ 0.03	0.25	S	R	S	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25	S	R	S	S	S	S	R
≤ 0.03	≥ 4.0	S	R	S	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25	S	S	R	S	S	S	R
≤ 0.03	0.25	S	R	S		S	S	S
≤ 0.03	0.25	S	R	R	S	S	S	S
≤ 0.03	0.5	S	R	S	S	S	S	
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25	S	S	R	S	S	S	R
≤ 0.03	0.25	S	S	R	R	R	S	S
≤ 0.03	0.25	S	R	S	S	S	S	S
≤ 0.03	0.25	S	R	S	R	R	S	S
≤ 0.03	0.25	S	S	R	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25		R	S				S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	≥ 4.0	S	R	R	S	S	S	R
≤ 0.03	0.25	S	S	R	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	≥ 4.0	S	S	R	S	S	S	R
≤ 0.03	0.25	S	S	R	R	R	S	S
≤ 0.03	0.25	S	S	S	S	S	S	R

Isolate	MLST	Spa	MIC VA	MIC DX	MIC CI	Disc TR	MIC TR	MIC SXT
6330	105	002	1	≤ 1.0	≥ 8.0	24		≤ 10.0
6441	22	223	≤ 0.5	≤ 1.0	≤ 0.5	6		≥ 320.0
6779	1153	903	≤ 0.5	≤ 1.0	≤ 0.5	26		≤ 10.0
6826	6	304	≤ 0.5	≤ 1.0	≤ 0.5	23		≤ 10.0
7211	4131	034	≤ 0.5	≥ 16.0	≤ 0.5	-		≤ 10.0
7261	1232	034	≤ 0.5	≥ 16.0	≤ 0.5	25		≤ 10.0
7346	8015	026	≤ 0.5	≤ 1.0	≤ 0.5	21		≤ 10.0
7435	8018	442		≤ 1.0	≥ 8.0	22		≤ 10.0
7450	6	304	≤ 0.5	≤ 1.0	≤ 0.5	22		≤ 10.0
7473	97	2770	1	≥ 16.0	2	19		≤ 10.0
7761	30	019	1	≥ 16.0	≤ 0.5	20		≤ 10.0
8148	1535	084	1	≤ 1.0	1	21		≤ 10.0
8178	5544	1081	1	≥ 16.0	≥ 8.0	21		≤ 10.0
8244	8	008	1	≤ 1.0	≤ 0.5	22		≤ 10.0
8349	8	008	≤ 0.5	≤ 1.0	≥ 8.0	21		≤ 10.0
8413	30	363	1	≤ 1.0	4	6		≥ 320.0
8578	5	1062	1	≤ 1.0	≤ 0.5	6		≥ 320.0
8852	6627	304	1	≤ 1.0	≤ 0.5	22		≤ 10.0
8935	1	127	1	≥ 16.0	≤ 0.5	21		≤ 10.0
9038	8016	304	1	≤ 1.0	≤ 0.5	23		≤ 10.0
9601	7119	132	≤ 0.5	≤ 1.0	2	23		≤ 10.0
9941	22	790	1	≤ 1.0	≤ 0.5	-		≤ 10.0
302760	22	2251	1	≤ 1.0	≤ 0.5	6		≥ 320.0
502760	1	127	1	≥ 16.0	≤ 0.5	17		≤ 10.0

Legend: MIC is in mg/L. VA: vancomycin, DX: doxycycline, CI: ciprofloxacin, TR: trimethoprim, SXT: trimethoprim/sulfamethoxazole, SF: sulphonamides, RA: rifampicin, CL: clindamycin, MP: mupirocin. Res = resistance. S/R: susceptible/intermediate/resistant. *etest MIC: 6mg/L.

MIC RA	MIC CL	Res VA	Res DX	Res CI	Res TR	Res SXT	Res RA	Res CL
≤ 0.03	≥ 4.0	S	S	R	S	S	S	R
≤ 0.03	0.25	S	S	S	R	R	S	S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	≥ 4.0	S	R	S		S	S	R
≤ 0.03	≥ 4.0	S	R	S	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25		S	R	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	R
≤ 0.03	0.25	S	R	R	S	S	S	S
≤ 0.03	0.25	S	R	S	S	S	S	S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.5	S	R	R	S	S	S	
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.5	S	S	R	S	S	S	S
≤ 0.03	0.25	S	S	R	R	R	S	S
≤ 0.03	0.25	S	S	S	R	R	S	S
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25	S	R	S	S	S	S	R
≤ 0.03	0.25	S	S	S	S	S	S	S
≤ 0.03	0.25	S	S	R	S	S	S	S
≤ 0.03	0.25	S	S	S		S	S	S
≤ 0.03	0.25	S	S	S	R	R	S	S
≤ 0.03	0.25	S	R	S	S	S	S	R

Table S2. Characteristics of isolates of patients that were treated with systemic antibiotics

Isolate	Treatment regimen/ duration	MIC/ susceptibility resistance genes DX	MIC/ susceptibility resistance genes VA	Disk zone/ susceptibility resistance genes TR
7346	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	21 / S -
0037	TR RA MP / 7	≥ 16.0 / R tet(K)	≤ 0.5 / S -	24/ S -
2654	DX RA MP / 7	≤ 1.0 / S -	1 / S -	23 / S -
502760	SXT RA MP / 14	≥ 16.0 / R tet(L)	1 / S -	17 / S -
3025	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	22 / S -
5606	SXT RA MP / 7	≤ 1.0 / S -	1 / S -	27 / S -
6118	TR RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	23 / S -
7435	TR RA MP / 7	≤ 1.0 / S -	- / - -	- / S -
7473	TR RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	19 / S -
7761	SXT RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	20 / S -
8852	SXT RA MP / 7	≤ 1.0 / S -	1 / S -	22 / S -
0507	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	- / R -
1475	DX RA MP / 14	≤ 1.0 / S -	1 / S -	6 / R -
2285	DX RA MP / 14	≤ 1.0 / R -	1 / S -	20 / S -
3460	SXT RA MP / 7	≤ 1.0 / R -	1 / S -	21 / - -
5940	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	- / R -
8413	DX RA MP / 7	≤ 1.0 / S -	1 / S -	6 / R dfrG
2213	SXT RA MP / 7	≥ 16.0 / R	1 / S	21 / S

MIC/ susceptibility SXT resistance genes SF	MIC/ susceptibility resistance genes RA	MIC/ susceptibility resistance genes CL	Outcome*
≤ 10.0 /S -	≤ 0.03/ S -	0.25/ R erm(C)	Failure
≤ 10.0 / S -	≤ 0.03/ S -	0.25 / S -	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(A)	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(A)	Failure
≤ 10.0 / S -	≤ 0.03 / S -	≥ 4.0 / R erm(C)	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Failure
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Failure
160 / R -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
80 / R -	≤ 0.03 / S -	0.25 / S -	Success
≥ 320.0 / R -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S	≤ 0.03 / S	0.25 / S	Success

Isolate	Treatment regimen/ duration	MIC/ susceptibility resistance genes DX	MIC/ susceptibility resistance genes VA	Disk zone/ susceptibility resistance genes TR
		tet(K)	-	-
2223	SXT RA MP / 7	≤ 1.0 / S -	1 / S -	25 / S -
2392	CL RA MP / 14	≤ 1.0 / S -	≤ 0.5 / S -	21 / S -
302760	CL RA MP / 7	≤ 1.0 / S -	1 / S -	6 / R -
2879	TR RA MP / 7	≤ 1.0 / S -	1 / S -	23 / S -
3260	DX RA MP / 7	≤ 1.0 / S -	1 / S -	22 / S -
3488	CL RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	19 / S -
3839	TR RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	19 / S -
4998	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	19 / S -
5308	DX RA MP / 7	≤ 1.0 / S -	1 / S -	21 / S -
6826	DX RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	23 / S -
7211	SXT RA MP / 14	≥ 16.0 / R tet(K)	≤ 0.5 / S -	- / S -
7261	SXT RA MP / 7	≥ 16.0 / R tet(K)	≤ 0.5 / S -	25 / S -
7450	TR RA MP / 7	≤ 1.0 / S -	≤ 0.5 / S -	22 / S -
8178	SXT RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	21 / S -
8244	VA CL MP / 7	≤ 1.0 / S -	1 / S -	22 / S -
8935	SXT RA MP / 7	≥ 16.0 / R tet(K)	1 / S -	21 / S -

Legend: Treatment duration is in days. MIC is in mg/L. Disc zone is in millimetres. VA: vancomycin, RA: rifampicin, CL: clindamycin, MP: mupirocin. S/I/R: susceptible/intermediate/resistant.

MIC/ susceptibility SXT resistance genes SF	MIC/ susceptibility resistance genes RA	MIC/ susceptibility resistance genes CL	Outcome*
-	-	-	
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≥ 320.0 / R -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(A)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	≥ 4.0 / R erm(C)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(A)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	≥ 4.0 / R erm(A)	Success
≤ 10.0 / S -	≤ 0.03 / S -	≥ 4.0 / R erm(A)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.5 / I -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / S -	Success
≤ 10.0 / S -	≤ 0.03 / S -	0.25 / R erm(C)	Success

DX: doxycycline, TR: trimethoprim, SXT: trimethoprim/sulfamethoxazole, SF: sulphonamides,

*Success = successful decolonization. Failure = failure of eradication treatment.

Table S3. Point mutations associated with ciprofloxacin and rifampicin resistance and MICs.

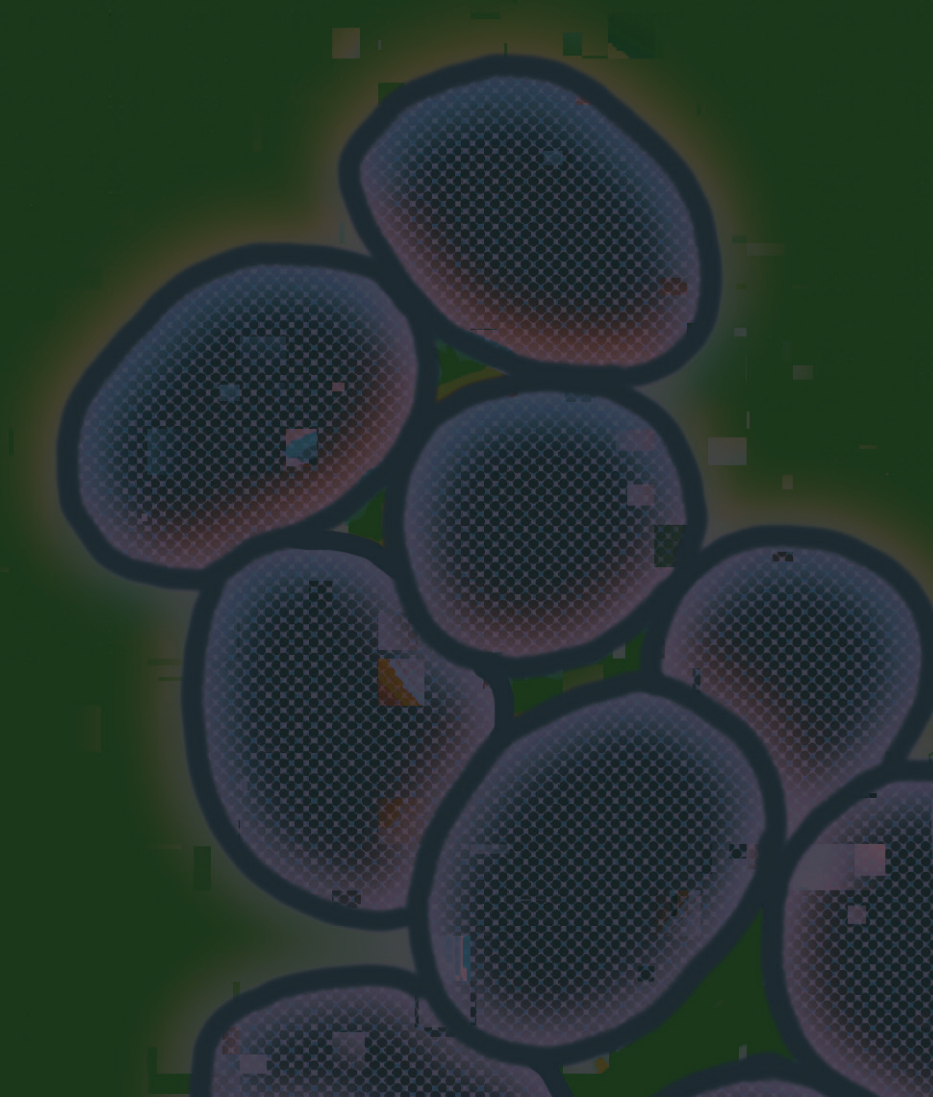
Isolate	Outcome	MIC ciprofloxacin	Ciprofloxacin-resistance associated mutations	MIC rifampicin	Rifampicin-resistance associated mutations
37	Failure	2	S80F grlA	≤ 0.03	D471Y rpoB
507	Success	≤ 0.5		≤ 0.03	
690	Success	≤ 0.5		≤ 0.03	
720	Success	2	S80F grlA	≤ 0.03	
1475	Success	≤ 0.5		≤ 0.03	
2213	Success	≤ 0.5		≤ 0.03	
2223	Success	≤ 0.5		≤ 0.03	
2285	Success	≤ 0.5		≤ 0.03	
2315	Success	1		≤ 0.03	
2392	Success	≤ 0.5		≤ 0.03	
2478	Success	1		≤ 0.03	
2654	Failure	2	S80F grlA	≤ 0.03	
2673	Success	≤ 0.5		≤ 0.03	
2703	Success	≥ 8.0	S80F grlA S84L gyrA	≤ 0.03	
2855	Success	≤ 0.5		≤ 0.03	
2866	Success	1	S80F grlA	≤ 0.03	
2879	Success	2	S80F grlA	≤ 0.03	
2905	Success	4	S80F grlA S84L gyrA	≤ 0.03	
2970	Success	≤ 0.5		≤ 0.03	
2980	Success	≤ 0.5		≤ 0.03	
3025	Failure	2	S80F grlA	≤ 0.03	
3260	Success	≤ 0.5		≤ 0.03	
3460	Success	≤ 0.5		≤ 0.03	
3464	Success	≤ 0.5		≤ 0.03	
3488	Success	≤ 0.5		≤ 0.03	
3839	Success	≥ 8.0	S84L gyrA S80Y grlA	≤ 0.03	
4211	Success	≥ 8.0	S84L gyrA S80Y grlA	≤ 0.03	
4998	Success	≤ 0.5	I45M grlA	≤ 0.03	
5308	Success	≤ 0.5		≤ 0.03	
5606	Failure	≥ 8.0	S80F grlA S84L gyrA P585S grlB	≤ 0.03	

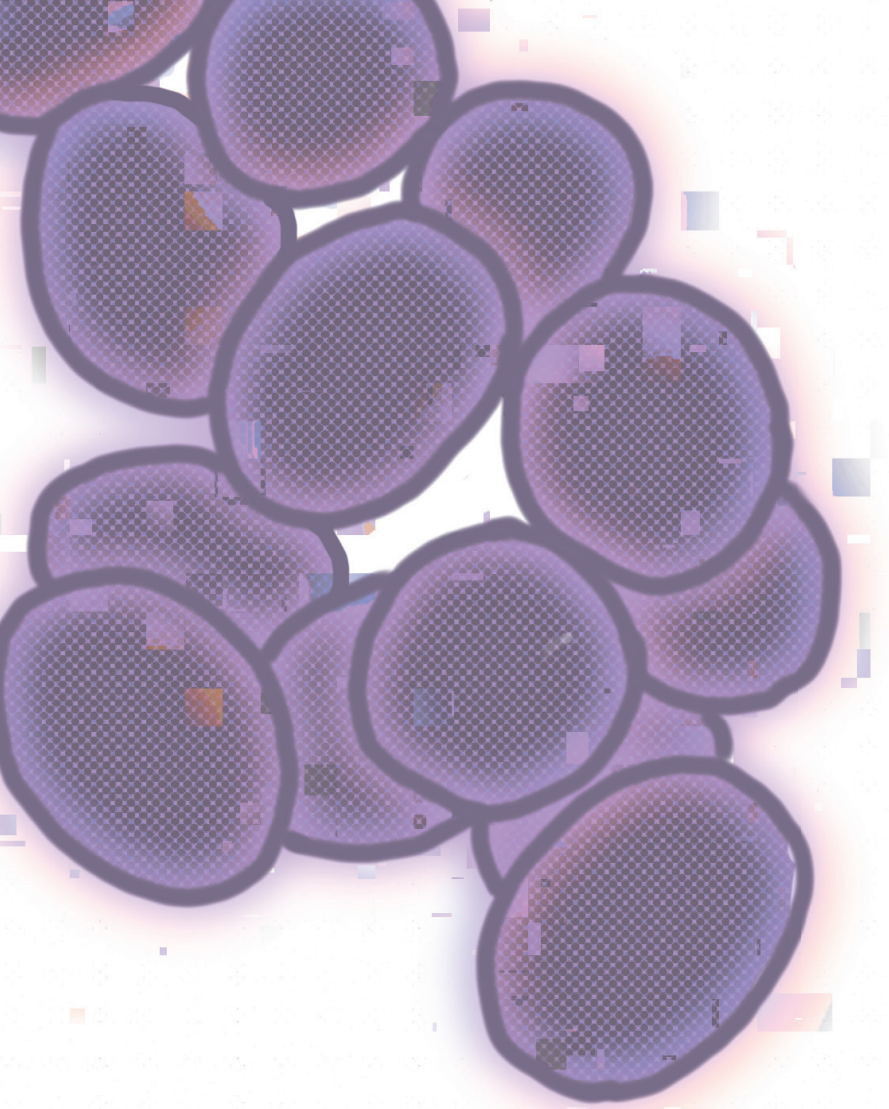
Isolate	Outcome	MIC ciprofloxacin	Ciprofloxacin-resistance associated mutations	MIC rifampicin	Rifampicin-resistance associated mutations
5940	Success	≥ 8.0	S80F grlA S84L gyrA	≤ 0.03	
6118	Failure	≤ 0.5		≤ 0.03	
6330	Success	≥ 8.0	S80y grlA S84L gyrA E84G grlA	≤ 0.03	
6441	Success	≤ 0.5		≤ 0.03	
6779	Success	≤ 0.5		≤ 0.03	
6826	Success	≤ 0.5		≤ 0.03	
7211	Success	≤ 0.5		≤ 0.03	
7261	Success	≤ 0.5		≤ 0.03	
7346	Failure	≤ 0.5	I45M grlA	≤ 0.03	
7435	Failure	≥ 8.0		≤ 0.03	
7450	Success	≤ 0.5		≤ 0.03	
7473	Failure	2	S80F grlA	≤ 0.03	I527L rpoB
7761	Failure	≤ 0.5		≤ 0.03	
8148	Success	1		≤ 0.03	
8178	Success	≥ 8.0	S80F grlA S84L gyrA E84G grlA	≤ 0.03	
8244	Success	≤ 0.5		≤ 0.03	
8349	Success	≥ 8.0	S80F grlA S84L gyrA	≤ 0.03	
8413	Success	4	S80F grlA S84L gyrA	≤ 0.03	
8578	Success	≤ 0.5		≤ 0.03	
8852	Failure	≤ 0.5		≤ 0.03	I527L rpoB
8935	Success	≤ 0.5		≤ 0.03	
9038	Success	≤ 0.5		≤ 0.03	
9601	Failure	2	S80F grlA I45M grlA	≤ 0.03	I527L rpoB
9941	Success	≤ 0.5		≤ 0.03	
302760	Success	≤ 0.5		≤ 0.03	
502760	Failure	≤ 0.5		≤ 0.03	

Legend: MIC is in mg/L.

Part 2

Challenges in *Staphylococcus aureus* bacteremia management





Chapter 6

Global differences in the management of *Staphylococcus aureus* bacteremia: no international standard of care

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Abstract

Background

Despite being the leading cause of mortality from bloodstream infections worldwide, little is known about regional variation in treatment practices for *Staphylococcus aureus* bacteremia (SAB). The aim of this study was to identify global variation in management, diagnostics, and definitions of SAB.

Methods

During a 20-day period in 2022, physicians throughout the world were surveyed on SAB treatment practices. The survey was distributed through listservs, e-mails, and social media.

Results

In total, 2031 physicians from 71 different countries on 6 continents (North America [701, 35%], Europe [573, 28%],Asia [409, 20%], Oceania [182, 9%], South America [124, 6%], and Africa [42, 2%]) completed the survey. Management-based responses differed significantly by continent for preferred treatment of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) bacteremia, use of adjunctive rifampin for prosthetic material infection, and use of oral antibiotics ($P < .01$ for all comparisons). The 18F-FDG PET/CT scans were most commonly used in Europe (94%) and least frequently used in Africa (13%) and North America (51%; $P < .01$). Although most respondents defined persistent SAB as 3–4 days of positive blood cultures, responses ranged from 2 days in 31% of European respondents to 7 days in 38% of Asian respondents ($P < .01$).

Conclusions

Large practice variations for SAB exist throughout the world, reflecting the paucity of high-quality data and the absence of an international standard of care for the management of SAB.

Introduction

Staphylococcus aureus is the leading cause of mortality by bloodstream infections worldwide [1], and methicillin resistant *S. aureus* (MRSA) is the leading cause of mortality attributable to antimicrobial resistance [2]. Despite its global distribution and an incidence of approximately 30 per 100 000 person-years [3, 4], the optimal approach to *S. aureus* bacteremia (SAB) is poorly understood. Despite the fact that SAB has been a major theme in the medical literature for decades, basic treatment elements such as the optimal antibiotic regimen, the role of adjunct and oral antibiotics, the optimal treatment duration, and the definition of persistent SAB remain fundamentally unknown. Even less is known about global differences in treatment practices for SAB.

The aim of this study was to identify global variation in management, diagnostics, and definitions of SAB. To do this, we used a variety of social media platforms to reach a large number of clinicians throughout the world for a survey on SAB treatment practices.

Methods

Survey development and distribution

We conducted this study on geographic practice variation in SAB by modifying a recently developed survey that was deployed in five European countries [5]. The modified survey was tested among an independent expert panel and adjusted where appropriate. The survey focused on unsettled aspects of the disease in clinical practice: first-choice antimicrobial agents, intravenous to oral switch of antimicrobial therapy, treatment duration, the use of 18F-fluoro-deoxyglucose positron emission tomography/computed tomography (PET/CT) (18F-FDG PET/CT) scan, and the definition of persistent SAB (Supplementary Appendix 1). When relevant, questions were provided separately for both methicillin-susceptible *S. aureus* (MSSA) and MRSA bacteremia. The survey was anonymous and voluntary. Country of practice was asked to determine geographic region and subsequently respondents were grouped by continent. The survey was developed in English. Target respondents included infectious diseases, clinical microbiology and internal medicine physicians (both adults and pediatrics) treating SAB patients throughout the world. The survey was distributed through a public URL link on listservs, e-mails, Twitter, and WeChat. Respondents were asked to share the survey link with their professional network. The link was accessible between 2 November and 22 November 2022.

Ethical approval

Given the anonymous and voluntary aspects of the survey, a declaration of exemption was issued by the institutional review board of Duke University.

Definitions

Uncomplicated bacteremia was defined as SAB that was not community-acquired, with <48 hours of positive blood cultures under appropriate antibiotic treatment, and no signs of metastatic infections. Oral switch therapy was defined as prescribing at least part of the treatment course orally. Both definitions were provided with the relevant questions. The estimated percentage of SAB patients in whom oral switch therapy was used was defined as never or uncommonly (<20% of SAB patients), sometimes (20%–60% of SAB patients) or frequently (>60% of SAB patients). All questions concerning antibiotic treatment assumed that the isolate was susceptible to the drug.

Data collection and management

Study data were collected and managed using Research Electronic Data Capture (REDCap) tools hosted at Duke University [6]. Respondents who completed 0 or 1 question only were removed from the analysis, as well as respondents that did not enter their country of practice. In order to remove potential non-targeted respondents, records were screened for straightliners (respondents that failed to differentiate between response alternatives by, for example, answering always only the first answer, or only the "other" option to every multiple- choice question) and for nonsensical answers to open-ended questions. Because the survey was distributed through different listservs and social media, the number of times the public survey URL link was opened was used to provide the best estimation of the response rate.

Statistical analysis

Descriptive statistics were used to summarize the data. Data were presented as percentages or proportions of the number of respondents that answered the question for categorical variables, and as medians plus interquartile range (IQR) for continuous variables. Pearson Chi^2 tests were performed to analyze differences between continents. All analyses were carried out using SPSS statistics version 28.0.1.1 (IBM Corporation, Armonk, New York, USA).

Results

A total of 2229 individual survey responses were obtained. The URL link was opened 5679 times (response rate 39%). Nine percent (198/2229) of records were removed from the analyses because of completion of ≤1 questions (88/2229, 4%) or not entering the country of practice (110/2229, 5%). No non-targeted responses were identified. The remaining survey records of 2031 respondents from 71 different countries on 6 continents (North America [701, 35%], Europe [573, 28%], Asia [409, 20%], Oceania [182, 9%], South America [124, 6%], and Africa [42, 2%]) were included in the analysis (Figure 1). Respondents stated they were physicians in adult infectious diseases (74%), clinical microbiology (10%), internal medicine (6%), and pediatric infectious diseases (5%). Thirteen percent of respondents were still in training, and 44% had been registered as a consultant for more than 10 years.

Antimicrobial management of SAB

Antibiotic treatment for SAB differed significantly between continents (Figure 2). For MSSA bacteremia, cefazolin was the first-choice antibiotic treatment in North America (78% of respondents), whereas anti-staphylococcal penicillins were preferred in all other continents (51%–82%; $P < .01$) (Figure 2A). For MRSA bacteremia, vancomycin was the preferred first-choice antibiotic agent in all continents, but with a broad range of 53%–97% of respondents. Daptomycin was identified as the first-choice antibiotic agent for MRSA bacteremia in 23% of European respondents but in <10% of respondents of all other continents (Figure 2B; $P < .01$ for all comparisons above.)

Adjunctive rifampin

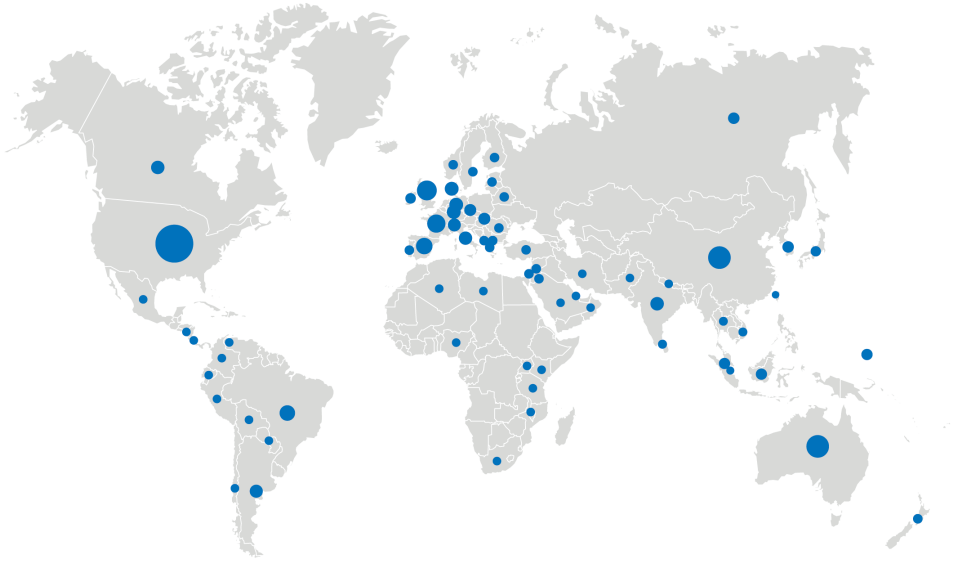
The practice of adding adjunctive rifampin in cases of SAB associated with infected prosthetic material was most frequently reported in Europe: 94% of European respondents would add it in at least 1 of the listed prosthetic material infections (cardiac device, endovascular device, joint prosthesis, prosthetic heart valve, and/or spondylodesis material infection). In Oceania and Africa rifampin was least often used in SAB patients with infected prosthetic material: 26% and 38% never added rifampin for this indication, respectively (Figure 2C).

Oral switch therapy

The estimated percentage of SAB patients in whom oral therapy was used was lowest in North America, where 76% of physicians indicated that they never or uncommonly used oral switch antibiotic therapy. Acceptance of oral therapy was highest in Europe, where 55% of physicians indicated that they used it frequently in their SAB patients

(Figure 3A). The majority of respondents from all continents except Oceania (48%) indicated that they would use oral switch therapy in uncomplicated SAB (57%–71%). Over half (54%–66%) of respondents from every continent identified patients with SAB originating from skin or soft tissue infection as a suitable patient group for safe oral switch therapy. By contrast, respondents differed widely on their views of the acceptability of oral therapy for SAB associated with spondylodiscitis, ranging from 19% in Africa to 60% in Oceania (Table 1). Source control and absence of a central nervous system infection were the only criteria for oral switch therapy for which there was broad agreement among respondents (79% and 69%, respectively) (Supplementary Table 1).

Figure 1. Global distribution of survey respondents. Respondents per country: 71 unique countries participated, and participation ranged from 1 to 654 respondents per country.



Treatment duration

The most commonly identified durations of therapy between geographic regions for SAB-associated syndromes were similar. Thus, the majority of respondents from each continent indicated the same duration of treatment for native valve endocarditis (6 weeks), septic arthritis (4 weeks), and spondylodiscitis (6 weeks). Despite these similarities in practice amongst the majority of practitioners across geographic regions, substantial "within-region" variation existed for these syndromes. For each infectious complication of SAB, individual respondents within each continent indicated longer and shorter durations of therapy (Figure 4).

The finding of blood cultures positive for *S. aureus* after 48–72 hours of appropriate therapy was identified as the most important reason to extend therapy duration in SAB patients beyond 2 weeks in all continents (range: 66% in South America to 90% in North America). Immunocompromised status was identified as an indication to extend antibiotic treatment beyond 2 weeks for most North American physicians (72%) but less than half (43%) of European physicians. By contrast, community acquisition of SAB was considered a reason to extend antibiotic treatment in only 20%–41% of physicians (Table 1; $P < .01$ for all above mentioned comparisons between continents).

18F-FDG PET/CT scan use

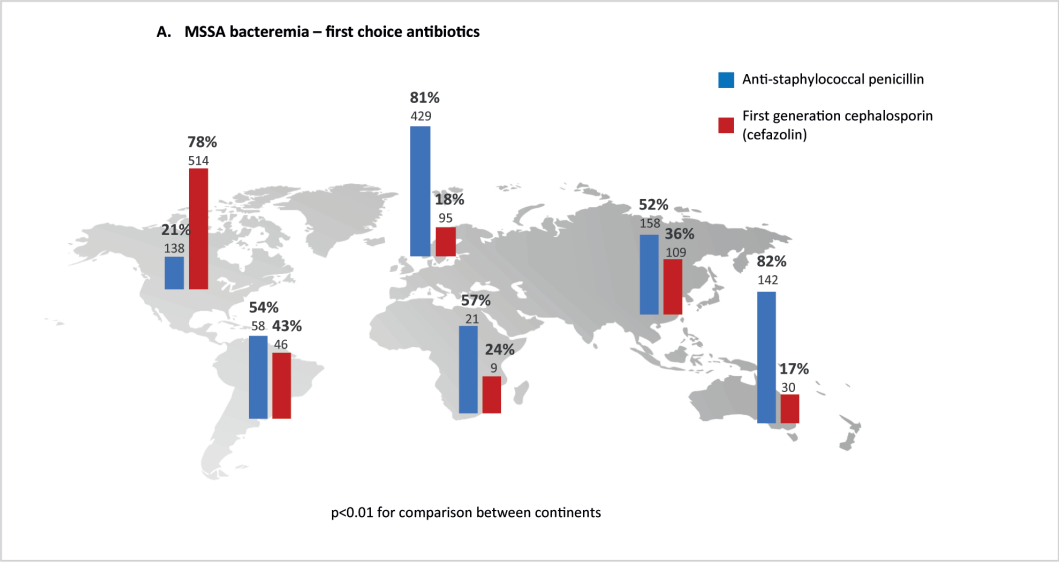
The availability, insurance coverage, and use of 18F-FDG PET/CT scans differed significantly between geographic regions (Table 1). All were highest in Europe and lowest in Africa. The direct availability of 18F-FDG PET/CT scans for SAB patients ranged from 9% in Africa and 29% in South America, to 78% in Europe. 18F-FDG PET/CT scans were used for SAB patients by 94% of European, 83% of Oceanian, 61% of South American, 57% of Asian, and 51% of North American physicians ($P < .01$ for both above mentioned comparisons between continents). Survey respondents indicating that they ordered 18F-FDG PET/CT scans in patients with SAB were asked to specify for which indications they did so. Globally, the most important and most agreed upon indication for 18F-FDG PET/CT scan in SAB was persistent bacteremia: 62%–70% of physicians in every continent ordered 18F-FDG PET/CT scans for this indication (Supplementary Table 2; $P = .66$).

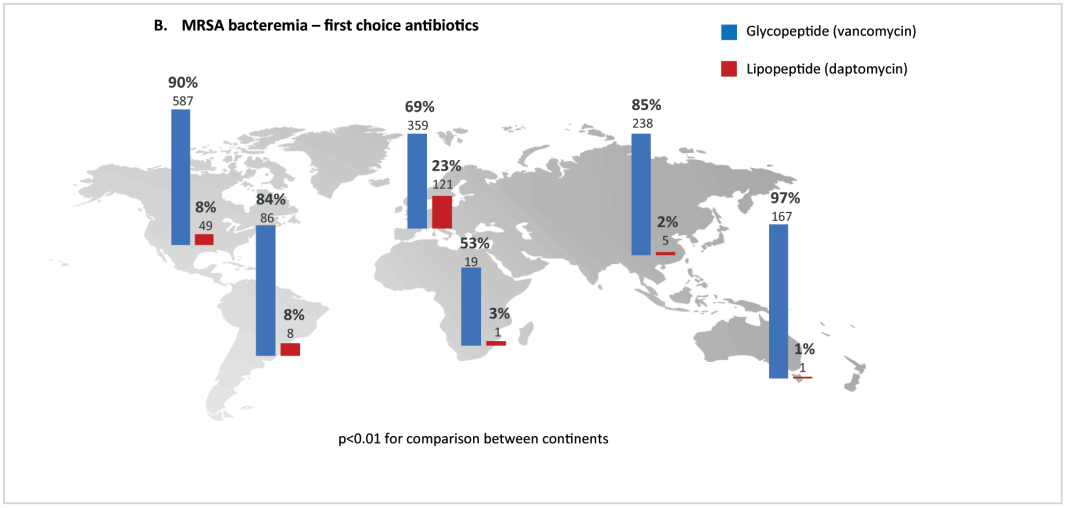
Persistent *S. aureus* bacteremia

The clinical definition of persistent SAB varied widely between continents. The most frequent definition of persistent SAB was a duration of at least 3-4 days of positive blood cultures despite appropriate treatment, identified by >33% of physicians in every continent. However, in Europe (31%) and South America (24%), a significant

minority of survey respondents indicated that persistent SAB was present after only 2 or more days of positive blood cultures. By contrast, 38% of Asian physicians indicated that seven or more days of positive blood cultures were required to constitute persistent SAB (Figure 3B). Almost all physicians indicated that they would order additional diagnostic testing in the setting of persistent SAB (79% in Africa, > 90% in all other continents), and a majority of physicians would also change their medical management (range 64% in Europe to 84% in North America; P < .01 for all above mentioned comparisons between continents) (Table 1).

Figure 2. Antibiotic treatment preferences for *S. aureus* bacteremia per continent. Percentage of total respondents of the question per continent, and count of respondents per continent. *Listed prosthetic materials: cardiac device, endovascular device, joint prosthesis, heart valve, and spondylodesis material.




B. MRSA bacteremia – first choice antibiotics
Glycopeptide (vancomycin)
Lipopeptide (daptomycin)
90%
587
8%
49
84%
86
8%
8
69%
359
23%
121
53%
19
3%
1
85%
238
2%
5
97%
167
1%
1
p<0.01 for comparison between continents

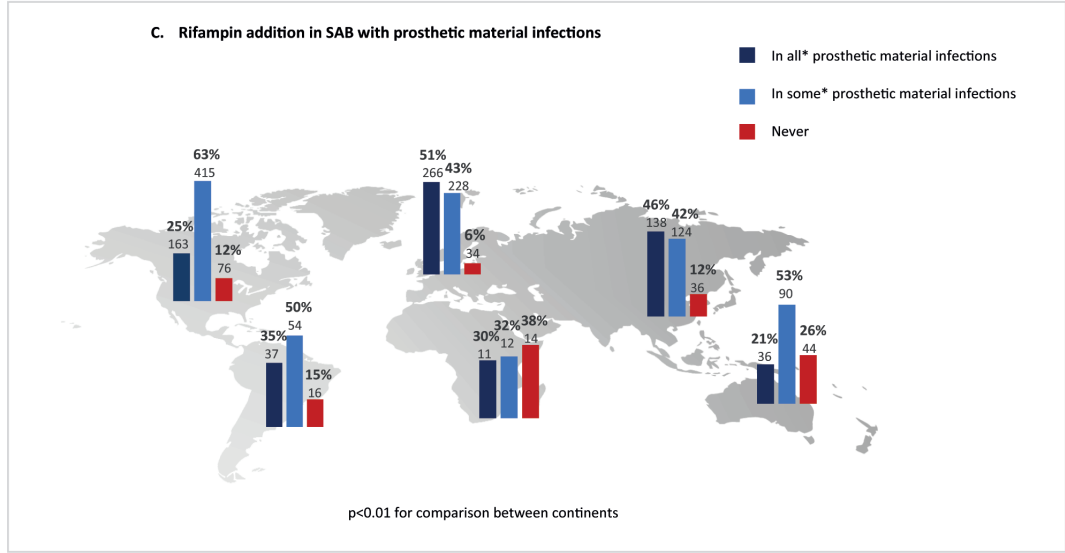

C. Rifampin addition in SAB with prosthetic material infections
In all* prosthetic material infections
In some* prosthetic material infections
Never
63%
415
25%
163
12%
76
50%
54
35%
37
15%
16
51%
266
43%
228
6%
34
30%
11
32%
12
38%
14
46%
138
42%
124
12%
36
53%
90
21%
36
26%
44
p<0.01 for comparison between continents

Figure 3. Oral switch therapy in SAB. Percentage (count) of total respondents of the question per continent. A, Estimated percentage of SAB patients per physician that are treated orally for at least part of the treatment course. B, Days of positive blood cultures while receiving adequate treatment to define persistent bacteremia, in *S. aureus* bacteremia. Abbreviation: SAB, *S. aureus* bacteremia.

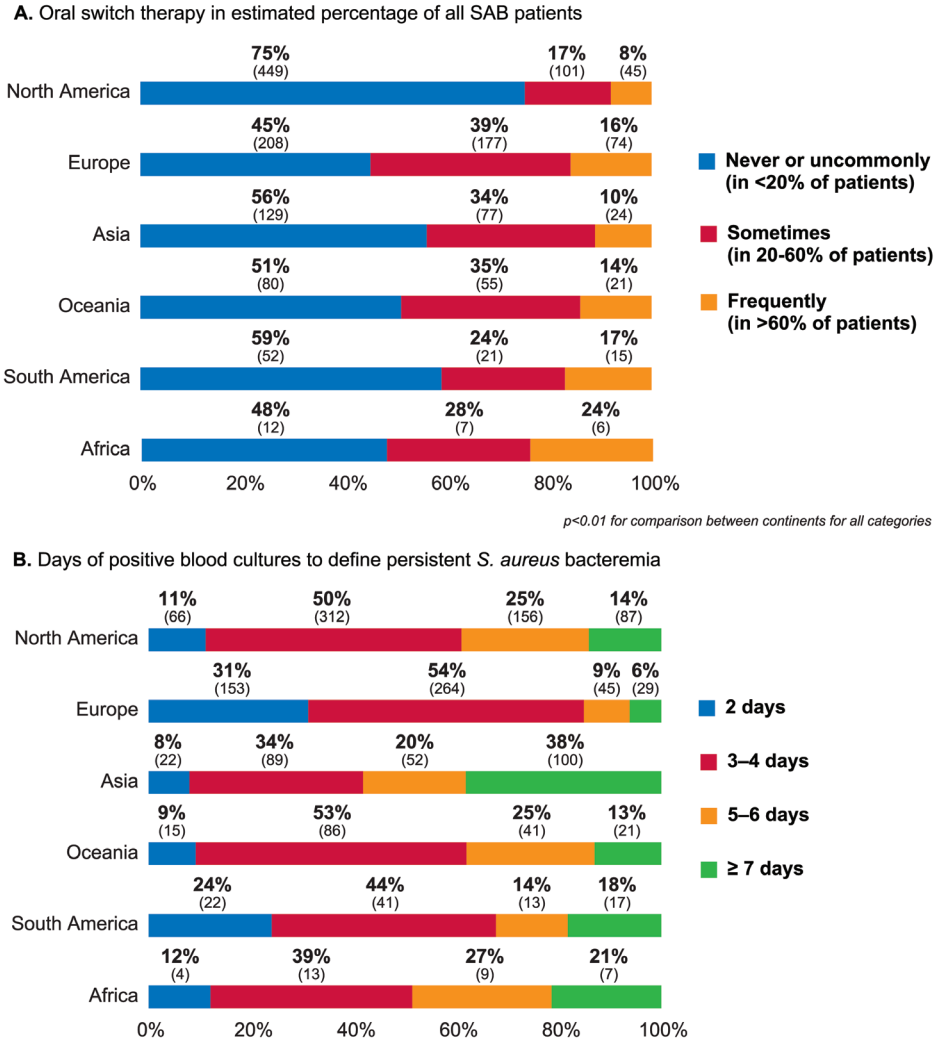
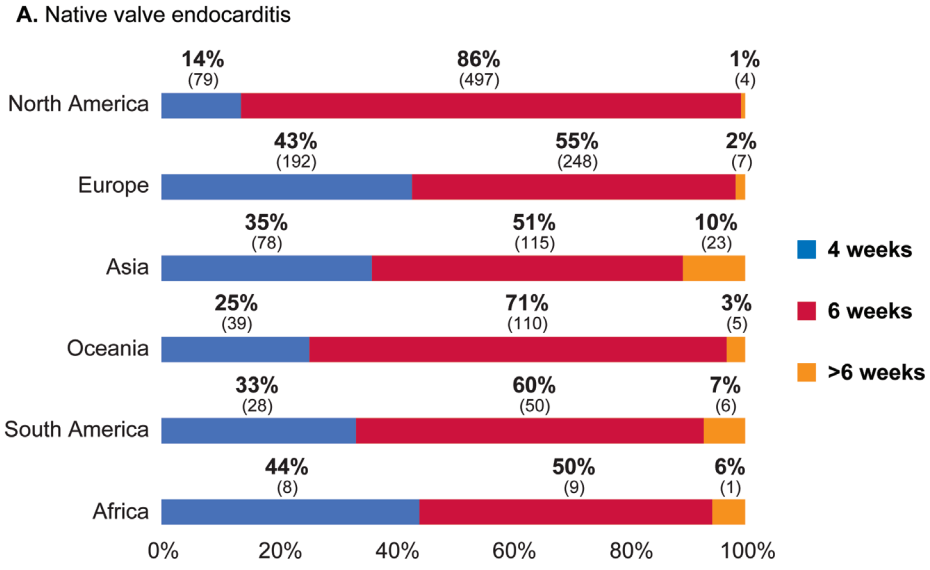


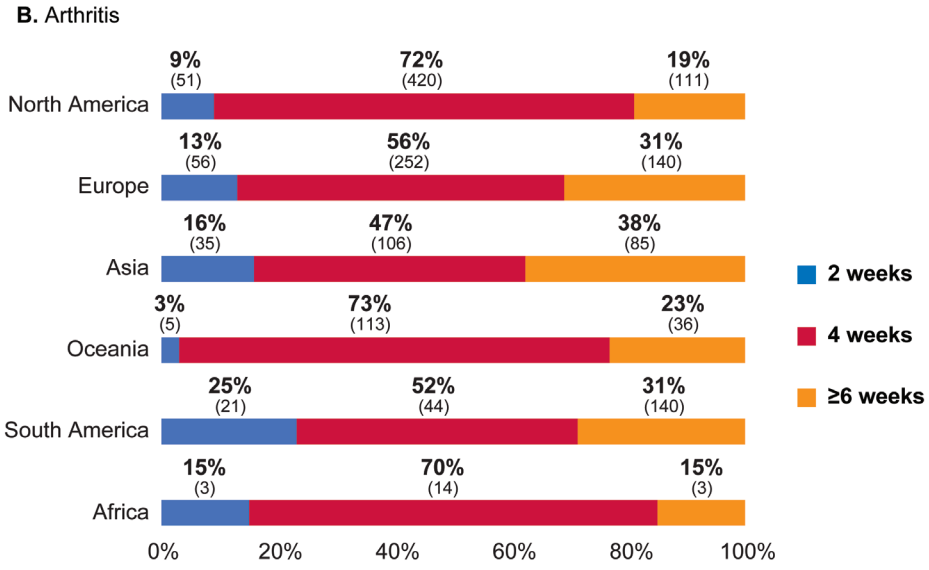
Table 1. Regional practice patterns for *S. aureus* bacteremia

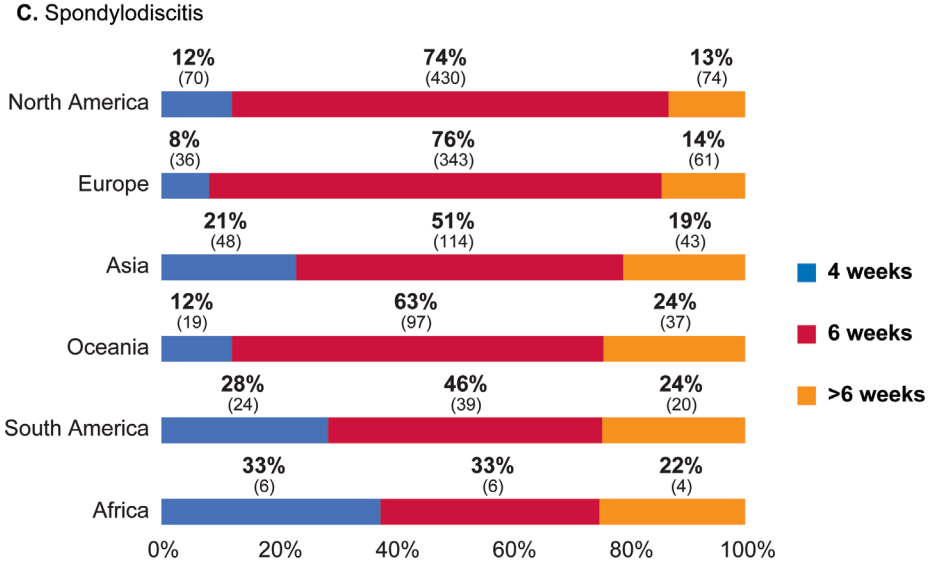
	Total	North America	Europe	Asia	Oceania	South America	Africa	
	N = 2031	N = 701	N = 573	N = 409	N = 182	N = 124	N = 42	p+
Cefazolin vs ASP in MSSAB								<0.01
Regarded equally effective	1470 (81.4)	557 (85.2)	416 (78.8)	235 (76.5)	148 (85.5)	86 (81.1)	28 (75.7)	
Reasons to extend therapy from 2 weeks to 4 weeks or more								<0.01
Positive BC 48-72h after start abx	1248 (84.0)	516 (89.7)	363 (83.3)	169 (76.1)	132 (86.8)	54 (65.9)	14 (77.8)	
Immuno-compromised	873 (59.5)	411 (72.0)	187 (43.2)	134 (62.9)	94 (63.1)	36 (44.4)	11 (55.0)	
Unknown portal of entry	787 (54.3)	386 (68.4)	178 (41.7)	102 (48.1)	87 (58.0)	28 (36.4)	6 (31.6)	
Fever 72h after first positive BC	746 (51.6)	331 (58.8)	203 (47.3)	94 (45.4)	78 (52.0)	32 (41.0)	8 (40.0)	
Community acquisition	460 (31.3)	231 (40.7)	96 (22.1)	44 (20.4)	71 (46.4)	14 (17.9)	4 (21.1)	
Oral switch therapy in different infection foci[1]								
Skin/soft tissue infection	922 (59.3)	330 (56.2)	305 (66.3)	140 (58.8)	85 (54.5)	47 (54.0)	15 (55.6)	0.01
Osteomyelitis	840 (54.0)	260 (44.3)	301 (65.4)	106 (44.5)	105 (67.3)	60 (69.0)	8 (29.6)	<0.01
Spondylodiscitis	659 (42.4)	155 (26.4)	275 (59.8)	84 (35.3)	94 (60.3)	46 (52.9)	5 (18.5)	<0.01
Prosthetic joint septic arthritis	610 (39.2)	144 (24.5)	260 (56.5)	75 (31.5)	89 (57.1)	37 (42.5)	5 (18.5)	<0.01
Prosthetic valve endocarditis	130 (8.4)	24 (4.1)	54 (11.7)	14 (5.9)	32 (20.5)	5 (5.7)	1 (3.7)	<0.01
All of the above	120 (7.7)	22 (3.7)	44 (9.6)	30 (12.6)	16 (10.3)	5 (5.7)	3 (11.1)	<0.01
18F-FDG PET/CT for SAB								
PET/CT readily available	829 (55.6)	278 (48.9)	341 (78.0)	106 (46.7)	78 (51.3)	24 (28.6)	2 (9.1)	<0.01
Covered by insurance for SAB	610 (42.2)	177 (33.2)	332 (77.4)	41 (18.2)	37 (24.3)	21 (25.0)	2 (9.1)	<0.01
PET/CT use in some/all patients	1009 (67.8)	293 (51.1)	409 (93.8)	125 (57.0)	124 (82.8)	50 (61.0)	4 (12.7)	<0.01
Never use PET/CT in SAB patients	479 (32.2)	281 (49.0)	27 (6.2)	96 (43.0)	26 (17.2)	32 (39.0)	17 (77.3)	<0.01
Available, but never use in SAB[2]	101 (12.2)	63 (22.7)	5 (1.5)	24 (23.1)	8 (10.3)	1 (4.2)	-	<0.01
Actions following diagnosis of persistent SAB								
Additional diagnostic testing	1610 (95.8)	605 (97.0)	484 (96.6)	242 (92.0)	161 (97.6)	91 (96.8)	27 (79.4)	<0.01
Change antibiotic management	1246 (74.7)	523 (83.5)	316 (63.8)	207 (80.2)	106 (64.6)	72 (76.7)	22 (68.8)	<0.01

N = the total number of respondents of the survey. Values are counts (%). Not all respondents answered every question, therefore the percentages represent the percentage of the total respondents of the continent who answered this question. Abbreviations: ASP, anti-staphylococcal penicillin; BC, blood culture; h, hours; MSSAB, methicillin-susceptible *Staphylococcus aureus*; PET/CT, positron emission tomography/computed tomography; SAB, *Staphylococcus aureus* bacteremia. [a]Results are still significant with P < .01 when continents with n ≤ 5 were excluded from analysis. [b]This represents the number and percent of respondents that indicated that PET/CT is readily available but still never use PET/CT in SAB patients.

Figure 4. Treatment duration for *S. aureus* bacteremia. Percentage (count) of total respondents of the question per continent. P < .01 for comparison between continents for all categories (χ2 test).




B. Arthritis
North America
9% (51)
72% (420)
19% (111)
Europe
13% (56)
56% (252)
31% (140)
Asia
16% (35)
47% (106)
38% (85)
Oceania
3% (5)
73% (113)
23% (36)
South America
25% (21)
52% (44)
31% (140)
Africa
15% (3)
70% (14)
15% (3)
0%
20%
40%
60%
80%
100%
2 weeks
4 weeks
≥6 weeks


C. Spondylodiscitis
North America
12% (70)
74% (430)
13% (74)
Europe
8% (36)
76% (343)
14% (61)
Asia
21% (48)
51% (114)
19% (43)
Oceania
12% (19)
63% (97)
24% (37)
South America
28% (24)
46% (39)
24% (20)
Africa
33% (6)
33% (6)
22% (4)
0%
20%
40%
60%
80%
100%
4 weeks
6 weeks
>6 weeks

Discussion

This study shows that even the most basic aspects of treating patients with SAB differ profoundly between geographic regions. This variation was most marked in fundamental aspects of decision-making for SAB treatment, including antibiotic choice for MSSA bacteremia, addition of rifampin for prosthetic device infections, and route of administration. An anti-staphylococcal penicillin was treatment of choice for MSSA bacteremia in Europe and Oceania but a distant second to cefazolin in North America. The evidence for superiority of either of the 2 is limited to cohort studies with conflicting results and with underrepresentation of complicated disease [7, 8], emphasizing the need for randomized trials.

The role of adjunctive rifampin in patients with prosthetic material infections also differed by continent. This controversy persists despite the availability of published society guidelines that recommend the use of rifampin in *S. aureus* infections involving prosthetic valves and arthroplasties [9, 10]. However, the recommendation to use rifampin for prosthetic valve infective endocarditis has a very limited evidence base [11]. Thus, well-designed randomized trials are needed to define any potential role of adjunctive rifampin in prosthesis-associated SAB. Importantly, the wide range of practices regarding the use of rifampin in this survey demonstrates the presence of the global equipoise necessary to ethically conduct such a trial. The practice of prescribing part of the treatment course for SAB with oral antibiotics was well accepted in all continents except North America, where only a minority of physicians would consider its routine use. This infrequent use of oral therapy in the United States may be due in part to a high prevalence of MRSA, the presence of a well-organized outpatient parenteral antimicrobial therapy system, or concerns related to medical malpractice. This lack of global consensus on the role of oral switch therapy is also reflected by the lack of consensus on which setting in which it should be considered. In fact, only the criteria of “source control” and “absence of central nervous system infection” were considered essential for oral switch by a clear majority. By contrast, all other listed criteria were regarded as essential by approximately half of the respondents—which implies that these were considered non-essential by the other half. Because oral switch therapy has potential to decrease the number of adverse drug events, catheter-associated problems and costs, and the fact that the survey respondents are in equipoise on the question, the need for a well-designed randomized trial seems clear. Current studies such as SABATO and SNAP might provide answers in the future [12, 13].

Broader global consensus existed for treatment duration of SAB. The worldwide similarity of respondents' views on treatment duration for SAB is noteworthy given that the data for this aspect of SAB is at least as limited as that for the treatment-related aspects outlined above for which there is significant controversy [14, 15]. Although the majority of surveyed physicians throughout the world treat complications of SAB such as endocarditis and osteoarticular infection for a similar duration, a portion of physicians in each continent will treat substantially longer or shorter. This finding suggests that factors influencing treatment duration decision-making may be provider-based and situational rather than simply geographical in nature.

18F-FDG PET/CT use

Our findings also indicate significant geographic variability in the use of 18F-FDG PET/CT as diagnostic tool in SAB, with broad use in Europe and Oceania being balanced by infrequency in other continents. Observational studies have reported that 18F-FDG PET/CT may impact management and reduce mortality in patients with SAB because of higher detection of metastatic foci [16, 17], although the reduced mortality may have been confounded by immortal time bias related to including patients dying before undergoing 18F-FDG PET/CT [18]. Obviously, the associated costs could be a reason to refrain from using 18F-FDG PET/CT in low- and middle-income regions, but this does not explain its highly variable use in high-income regions. Recently, a call to action was published in the United States, advocating for insurance coverage of 18F-FDG PET/CT use in SAB patients [19]. In order to reach that goal, high-quality studies including randomized trials of 18F-FDG PET/CT are warranted.

Definition of persistent SAB

The results of our survey suggest that the identification of persistent SAB may be therapeutically important, as it triggers additional diagnostic testing and changes in medical management for the majority of respondents. However, respondents generally disagreed on how to define it. Although 3–4 days was the most common identified definition of persistent SAB overall, all options in the range of 2–7 or more days were selected by respondents from each continent. Roughly one third of European respondents defined persistent SAB as only 2 days of bacteremia, although a similar portion of Asian respondents indicated that it occurred after 7 or more days. The prognostic significance of persistent SAB has been previously demonstrated [20–22]. Identifying a broadly accepted definition of persistent SAB would thus be helpful to optimize clinical decision-making, as well as to harmonize the terminology used in clinical research.

Perspective

The current study suggests that there is no global standard of practice for SAB. Striking differences were noted, both between and within continents, in what antibiotics were prescribed, and by what route. The lack of a global standard in the management of SAB stands in stark contrast to treatment of other syndromes of comparable lethality. For coronary artery disease, management has been largely standardized by guidelines based on data from randomized controlled trials [23–25]. Coincident with establishing these best treatment practices, the annual US mortality rate from coronary artery disease declined by 17.7% from 2005 to 2015 [26]. By contrast, the 1-month mortality for patients with SAB only decreased by 2.8% over the same time period [27]. The results of this study underscore one key fact: a global standard of care for SAB will be difficult to develop pending more definitive clinical trials data. Indeed, fewer than 3500 patients have been enrolled in published SAB randomized trials over the past 20 years (Supplementary Table 3). Factors other than robust clinical data, such as cultural differences, costs and availability of resources also influence management choices. However, without consensus on best practice, normative and cultural factors gain influence on for example antibiotic prescription behavior [28]. Multinational clinical trials such as the *Staphylococcus aureus* Network Adaptive Platform (SNAP) [13] are thus essential to standardize clinical definitions, identify treatment strategies, and improve patient outcomes of this common and frequently lethal infection.

Strengths and limitations

The current study illustrates the potential of using social media to understand global treatment practices and decision making. Although previous studies on physicians' management of SAB have been conducted [5, 29, 30], none were as extensive and on a global scale as this current study. Our study has several limitations. There were relatively low participation rates from South America and Africa. The respondents were not questioned about their local guideline and adherence to it, and for many countries no national guidelines were available. This made it impossible for us to consider the role of national guidelines in the present study. Given the fact that 71 countries were included in the survey, comparing differences between each of these countries was methodologically infeasible. Therefore, we limited the analyses to continents. We were unable to evaluate spatial clustering of infections. The survey was only available in English, which might have dissuaded non-English speaking physicians. Because the survey was distributed through listservs and social media, the exact number of recipients or proportion of physicians per country is unknown. Therefore, the true response rate is uncertain and could only be estimated by the ratio of the reported surveys and the number of times the URL link was opened.

Finally, because it was impossible to control who filled out the survey, respondents theoretically could have been non-physicians or could have completed the survey multiple times. However, because there was no incentive in responding, and we did not discover any nonsensical answers, this seems unlikely. Overall, the advantage of receiving feedback from over 2000 specialists from all over the world outweighs the potential disadvantages of the use of social media platforms.

Conclusion

Large practice variations for SAB exist throughout the world, reflecting the absence of an international standard of care for the management of patients with SAB. This article sets the stage and the agenda for multinational or global clinical trials and networks, to address the unresolved aspects of this devastating disease.

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Supplementary data

Appendix 1. Survey questions

In which country do you currently practice? _____

Which of the following best describes your primary area of medical specialty?

- o Clinical microbiology
- o Infectious diseases (adults)
- o Infectious diseases (pediatric)
- o Internal medicine
- o Other

How many years have you been registered as a consultant (i.e. medical specialist)?

- o Still in training for consultant
- o 0-10 years
- o 11-20 years
- o 21-30 years
- o More than 30 years

The following question refers to MSSA bacteremia

What is your first-choice initial antibiotic regimen in patients with confirmed monobacterial MSSA bacteremia without implanted prosthetic material provided the isolate is susceptible to the drug?

- □ Aminoglycoside, e.g. gentamicin
- □ Anti-staphylococcal penicillin, e.g. flucloxacillin dicloxacillin
- □ Carbapenem, e.g. meropenem
- □ Clindamycin
- □ First-generation cephalosporin, e.g. cefazolin
- □ Second-generation cephalosporin, e.g. cefuroxime
- □ Third-generation cephalosporin, e.g. ceftriaxone
- □ Fourth-generation cephalosporin, e.g. cefepime
- □ Fifth generation cephalosporin, i.e. ceftaroline
- □ Fluoroquinolone, e.g. levofloxacin
- □ Glycopeptide, e.g. vancomycin
- □ Linezolid
- □ Lipopeptide, e.g. daptomycin
- □ Piperacillin/tazobactam
- □ Rifampicin
- □ Trimethoprim/sulfamethoxazole
- □ Other

Do you consider first-generation cephalosporins (e.g. cefazoline) to have equivalent clinical effectiveness for MSSA bacteremia without central nervous system infection as anti-staphylococcal penicillins (e.g. flucloxacillin, dicloxacillin)?

- o Yes
- o No

Do you treat patients with *Staphylococcus aureus* bacteremia and the following types of infected prosthetic material which will not be removed with rifampicin as part of combination antibiotic therapy provided the isolate is susceptible to the drug? Mark all that apply.

- □ Cardiac device
- □ Endovascular graft
- □ Joint prosthesis
- □ Prosthetic heart valve
- □ Spondylodesis
- □ All of the above
- □ None of the above

The following question refers to MRSA bacteremia

What is your first-choice initial antibiotic regimen in patients with confirmed monobacterial MRSA bacteremia without implanted prosthetic material provided the isolate is susceptible to the drug?

- □ Aminoglycoside, e.g. gentamicin
- □ Clindamycin
- □ Fifth generation cephalosporin, i.e. ceftaroline
- □ Fluoroquinolone, e.g. levofloxacin
- □ Fosfomycin
- □ Glycopeptide, e.g. vancomycin
- □ Linezolid
- □ Lipopeptide, e.g. daptomycin
- □ Rifampicin
- □ Tetracycline, e.g. doxycycline
- □ Trimethoprim/sulfamethoxazole
- □ Other
- □ Combination therapy

The following questions refer to persistent bacteremia.

After how many days (-or more) of positive blood cultures with *S. aureus* despite adequate antibiotic therapy would you consider it a ‘persistent bacteremia’?

- o 2 days
- o 3 days
- o 4 days
- o 5 days
- o 6 days
- o 7 days
- o >7 days
- o Do not know

Would you order additional diagnostic testing in persistent bacteremia and if yes, after how many days of positive blood cultures?

- o Yes, after … days of adequate antibiotic therapy and persistent positive blood cultures
- o No, I would not order additional diagnostic testing in case of persistent bacteremia

What kind of additional diagnostic testing would you order? Mark all that apply.

- o Transthoracic echocardiography
- o Transesophageal echocardiography
- o CT- scan
- o PET-CT scan
- o MRI scan
- o Other: …..

Would you change medical management (e.g., change antibiotics, increase dose or duration of antibiotics; add 2nd antibiotic) in case of persistent bacteremia and if yes, after how many days of positive blood cultures?

- o Yes, after …….. days of adequate antibiotic therapy and persistent positive blood culture
- o No, I would not change medical management in case of persistent bacteremia

In case of MSSA persistent bacteremia: what would you change in terms of medical management? Mark all that apply.

- o Change antibiotic agents
- o Increase dose of antibiotics
- o Add 2nd (or 3rd) antibiotic agent
- o Prolong treatment
- o Other: …….

In case of MSSA persistent bacteremia: to what antibiotic regimen would you change?

- □ Aminoglycoside, e.g. gentamicin
- □ Anti-staphylococcal penicillin, e.g. flucloxacillin, dicloxacillin
- □ Carbapenem, e.g. meropenem
- □ Clindamycin
- □ First-generation cephalosporin, e.g. cefazolin
- □ Second-generation cephalosporin, e.g. cefuroxime
- □ Third-generation cephalosporin, e.g. ceftriaxone
- □ Fourth-generation cephalosporin, e.g. cefepime
- □ Fifth generation cephalosporin, i.e. ceftaroline
- □ Fluoroquinolone, e.g. levofloxacin
- □ Glycopeptide, e.g. vancomycin

- □ Linezolid
- □ Lipopeptide, e.g. daptomycin
- □ Piperacillin/tazobactam
- □ Rifampicin
- □ Trimethoprim/sulfamethoxazole
- □ Other

In case of MRSA persistent bacteremia: what would you change in terms of medical management? Mark all that apply.

- ○ Change antibiotic agents
- ○ Increase dose of antibiotics
- ○ Add 2nd (or 3rd) antibiotic agent
- ○ Other: …….

In case of MRSA persistent bacteremia: to what antibiotic regimen would you change?

- □ Aminoglycoside, e.g. gentamicin
- □ Clindamycin
- □ Fifth generation cephalosporin, i.e. ceftaroline
- □ Fluoroquinolone, e.g. levofloxacin
- □ Fosfomycin
- □ Glycopeptide, e.g. vancomycin
- □ Linezolid
- □ Lipopeptide, e.g. daptomycin
- □ Rifampicin
- □ Tetracycline, e.g. doxycycline
- □ Trimethoprim/sulfamethoxazole
- □ Other

The following questions refer to oral step-down therapy in MSSA/MRSA bacteremia.

Do you consider oral step-down antibiotic therapy in patients with uncomplicated *Staphylococcus aureus* bacteremia?

- o Yes
- o No

Do you consider oral step-down antibiotic therapy in patients with *Staphylococcus aureus* bacteremia and the following foci of infection? Mark all that apply.

- □ Brain abscess
- □ Central line infection
- □ Epidural abscess
- □ Native joint septic arthritis
- □ Native valve endocarditis
- □ Osteomyelitis
- □ Prosthetic joint septic arthritis
- □ Prosthetic valve endocarditis
- □ Skin- and soft tissue infection without abscess
- □ Urinary tract infection
- □ Vertebral osteomyelitis
- □ None of the above

In your opinion, which of the following criteria must a patient with *Staphylococcus aureus* bacteremia who is able to take oral medication fulfill to be eligible for oral step-down antibiotic therapy? Mark all that apply.

- □ Absence of central nervous system infection
- □ Absence of endovascular infection focus other than endocarditis
- □ Blood culture negativity 48-72 hours after initiation of adequate antibiotic treatment
- □ Blood culture negativity for at least 72 hours
- □ Defervescence within 72 hours after initiation of adequate antibiotic treatment
- □ Afebrile for at least the past 72 hours
- □ Hospital acquired bacteremia
- □ Initiation of adequate antibiotic treatment within 48 hours of blood culture collection
- □ PET-CT without signs of endocarditis and metastatic infections
- □ No evidence of metastatic foci (on clinical of radiologic examination, but radiological imaging is not required if not clinically indicated)
- □ Primary infection focus was line related or skin/soft tissue related
- □ Source control is achieved
- □ Transesophageal echocardiography (TEE) without signs of endocarditis
- □ Transthoracic echocardiography (TTE) without signs of endocarditis
- □ None of the above

If oral drugs are acceptable in your opinion, what is your most commonly prescribed antibiotic regimen for oral step-down therapy in patients with confirmed MSSA bacteremia without implanted prosthetic material provided the isolate is susceptible to the drug? *Choose only one answer, unless you routinely prescribe combination therapy. In that case mark all that apply.*

- □ Anti-staphylococcal penicillin, e.g. flucloxacillin, dicloxacillin
- □ Oral cephalosporin (e.g., cefalexin, cefadroxil)
- □ Clindamycin
- □ Fluoroquinolone, e.g. levofloxacin
- □ Fusidic acid
- □ Linezolid
- □ Macrolide, e.g. erythromycin
- □ Penicillin, e.g. amoxicillin
- □ Rifampicin
- □ Tetracycline, e.g. doxycycline
- □ Trimethoprim/sulfamethoxazole
- □ Probenicid
- □ Other

If oral drugs are acceptable in your opinion, what is your most commonly prescribed antibiotic regimen for oral step-down therapy in patients with confirmed MRSA bacteremia without implanted prosthetic material provided the isolate is susceptible to the drug? Choose only one answer, unless you routinely prescribe combination therapy. In that case mark all that apply.

- □ Clindamycin
- □ Fluoroquinolone, e.g. levofloxacin
- □ Fusidic acid
- □ Linezolid
- □ Macrolide, e.g. erythromycin
- □ Rifampicin
- □ Tetracyclin, e.g. doxycycline
- □ Trimethoprim/sulfamethoxazole
- □ Other

In what estimated percentage of the patients you treat for *Staphylococcus aureus* bacteremia, do you prescribe at least part of the treatment course orally (instead of prescribing IV antibiotics during the entire treatment course)?

- o 0% (I never treat patients with SAB with oral antibiotics, also not temporarily)
- o 1-20%
- o 21-40%
- o 41-60%
- o 61-80%
- o 81-100% (I treat almost every patient with SAB for at least part of the treatment with oral antibiotics)

The following questions refer to treatment duration.

How many weeks of antibiotic treatment (includes both IV and oral) would you prescribe in a patient with *Staphylococcus aureus* bacteremia without implanted prosthetic material and the following foci of infection?

	2 weeks	**4 weeks**	**6 weeks**	**>6 weeks**
Arthritis	○	○	○	○
Native valve endocarditis	○	○	○	○
Long bone osteomyelitis	○	○	○	○
Pneumonia without abscess	○	○	○	○
Septic thrombophlebitis	○	○	○	○
Spondylodiscitis without abscess	○	○	○	○

Would the following factors make you consider extending antibiotic therapy from 2 weeks to 4 weeks in a patient with *Staphylococcus aureus* bacteremia? Assume transesophageal echocardiography (TEE) does not show signs of endocarditis.

	Yes	No
Community-acquisition	○	○
Delay of 48 hours between sampling first positive blood culture and initiation of adequate antibiotic treatment	○	○
Fever at 72 hours after first positive blood culture	○	○
Positive blood cultures after 72 hours of adequate antibiotic treatment	○	○
Unknown portal of entry	○	○
Methicillin resistant *Staphylococcus aureus*	○	○
Age > 75 years	○	○
Immunocompromised patient	○	○

The following questions refer to PET-CT scan.

Is PET-CT readily available in your setting for investigation of SAB?

- Yes
- No

Is PET-CT covered by insurance / reimbursed for the indication of SAB?

- Yes
- No

In which situations do you use PET-CT in patients with *Staphylococcus aureus* bacteremia? Mark all that apply.

- I never use PET-CT in patients with SAB
- In all patients with SAB
- When SAB is community acquired
- In patients with MRSA bacteremia
- In patients with persistent fever >48h after adequate therapy
- In patients with persistent fever >72h after adequate therapy
- In patients with persistent bacteremia
- In patients >75 years old
- In patients with prosthetic joint material
- In patients suspected of endocarditis
- In patients with clinical signs of metastatic infection
- Other, please clarify: …..

Table S1. Criteria that must be fulfilled for oral switch therapy in *S. aureus* bacteremia

	Total	North America	Europe	Asia	Oceania	South America	Africa	
	N=1156	N=399	N=372	N=177	N=120	N=70	N=18	p
Source control achieved	914 (79.1)	325 (81.5)	307 (82.5)	125 (70.6)	87 (72.5)	57 (81.4)	13 (72.2)	<0.01
Absence of central nervous system infection	793 (68.6)	289 (72.4)	248 (66.7)	123 (69.5)	67 (55.8)	53 (75.7)	13 (72.2)	0.01
Negative blood culture 48-72h after start antibiotics	652 (56.4)	247 (61.9)	217 (58.3)	85 (48.0)	52 (43.3)	39 (55.7)	12 (66.7)	<0.01
Absence of endovascular focus	558 (48.3)	202 (50.6)	173 (46.5)	93 (52.5)	45 (37.5)	31 (44.3)	14 (77.8)	0.01
Afebrile for at least 72h	644 (55.7)	206 (51.6)	224 (60.2)	91 (51.4)	64 (53.3)	49 (70.0)	10 (55.6)	0.02
No evidence of metastatic foci	573 (49.6)	217 (54.4)	167 (44.9)	101 (57.1)	41 (34.2)	42 (60.0)	5 (27.8)	<0.01
Negative blood culture for at least 72h	602 (52.1)	220 (55.1)	182 (48.9)	99 (55.9)	63 (52.5)	31 (44.3)	7 (38.9)	0.2

Legend. Values are counts (% of respondents of region who answered the question). N = number of respondents that answered this question. p value refers to difference between continents.

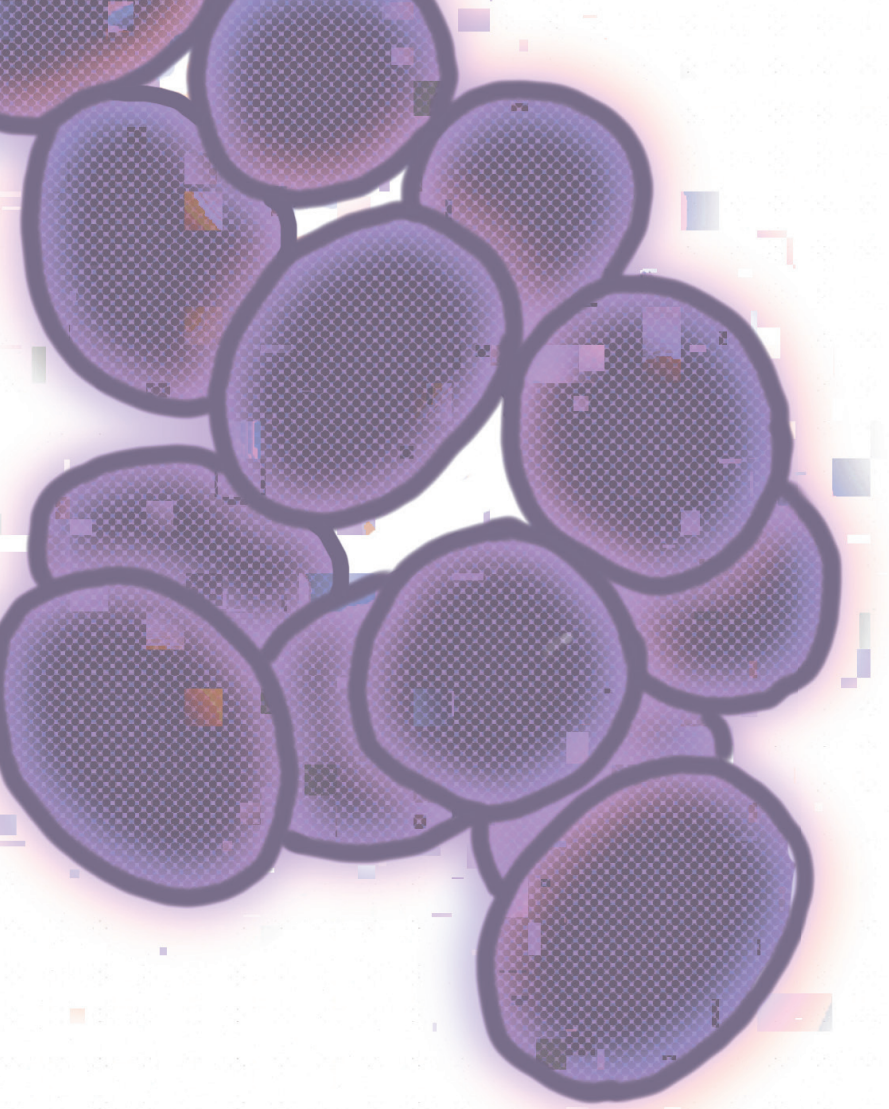
Table S2. Indications for 18F-FDG PET/CT use in *S. aureus* bacteremia patients

	Total N=993	North America N=292	Europe N=398	Asia N=125	Oceania N=124	South America N=50	p
Persistent bacteremia	666 (67.1)	203 (69.5)	269 (67.6)	84 (67.2)	78 (62.9)	31 (62.0)	0.66
Signs of metastatic infection	518 (52.2)	115 (39.4)	247 (62.1)	75 (60.0)	41 (33.1)	38 (76.0)	<0.01
Persistent fever 48-72h	367 (37.0)	95 (32.5)	169 (42.5)	43 (34.4)	38 (30.6)	22 (44.0)	0.03
Prosthetic joint material	279 (28.1)	60 (20.5)	144 (36.2)	32 (25.6)	25 (20.2)	18 (36.0)	<0.01
Suspected endocarditis	311 (31.3)	52 (17.8)	172 (43.2)	34 (27.2)	33 (26.6)	18 (36.0)	<0.01

Legend. Values are counts (% of respondents of region who answered the question). N = number of respondents that answered this question. p value refers to difference between continents. Africa was excluded here because 18F-FDG PET/CT was almost never used.

Table S3. Randomized controlled trials on *S. aureus* bacteremia patients in the past 20 years

Study	Year	Number of patients
Fowler NEJM	2006	246
Weems AAC	2006	63
Ruotsalainen J Int Med	2006	381
Rupp AAC	2007	40
Stryjewski BMC	2014	60
Paul BMJ	2015	91
Davis CID	2016	60
Xbiotech	2016	52
Thwaites Lancet	2017	758
Holland JAMA	2018	116
Pericas CMI	2018	15
Geriak AAC	2019	40
Fowler JCI	2020	116
Tong JAMA	2020	352
Cheng CID	2021	104
Pujol CID	2021	155
Kaasch ECCMID	2022	213
Holland IDWeek	2022	390
Total		**3252**



Chapter 7

Acute kidney injury in *Staphylococcus aureus* bacteremia

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Abstract

Acute kidney injury (AKI) is a frequent complication in patients with *Staphylococcus aureus* bacteremia (SAB), with a significant impact on patient management and outcome. This study aimed to provide insight in the proportion of patients with SAB that develop AKI, the risk factors for developing AKI in this population, and its reversibility. In this retrospective, multicenter cohort study, adult patients with SAB were eligible for inclusion. Patient characteristics, clinical variables, and laboratory results were retrieved from the electronic patient files. Primary outcome was development of AKI, defined as 1.5 times baseline creatinine. Secondary outcomes were reversibility of AKI and risk factors for AKI. A total of 315 patients with SAB were included, of whom 115/315 (37%) developed acute kidney injury. In 68/115 (59%), the AKI was reversible. If kidney function recovered, this occurred within 7 days in 56/68 (82%) of patients. In multivariable logistic regression analyses, independent risk factors for AKI were as follows: complicated SAB, use of diuretics, and hemodynamic instability. Development of AKI was associated with 30-day mortality (OR 3.9; CI 2.2–6.9; $p < 0.01$). Acute kidney injury is a frequent complication in patients with *Staphylococcus aureus* bacteremia. Considering the irreversibility in a relevant proportion of patients, future research into the underlying pathophysiology and potential interventions is warranted.

Introduction

Staphylococcus aureus is a major cause of bloodstream infections and is associated with high morbidity and mortality rates [1, 2]. Acute kidney injury (AKI) is a frequent complication in patients with *Staphylococcus aureus* bacteremia (SAB), with a significant impact on patient management and outcome [3, 4]. The etiology of AKI in SAB is diverse, including prerenal, toxic/drug-related, immune-mediated, tubulointerstitial nephritis (TIN), acute tubular necrosis (ATN), and postrenal pathophysiology. Despite the fact that acute kidney injury in patients with SAB is common, little is known about the proportion of patients with SAB that develop AKI, the risk factors for developing AKI in these patients, and its reversibility. The SAB patient population is heterogeneous, and the disease course varies greatly, from transient bacteremia in uncomplicated SAB to widespread infection and metastatic disease in complicated SAB [5]. Although likely on theoretical grounds, it is unknown whether the incidence, etiology, and outcome of AKI differ between complicated and uncomplicated SAB [6].

The aim of this study was to investigate the incidence of AKI in SAB, its reversibility, the risk factors for the development of AKI, and differences in disease course

between complicated and uncomplicated SAB. Additional knowledge of AKI in SAB may provide clinicians tools to predict risk of AKI in individual patients and support diagnostic and therapeutic management. Eventually, it could lead to initiation of intervention studies aimed at prevention or treatment of AKI in patients with SAB.

Methods

Study population

This multicenter retrospective cohort study was performed in one academic and two large teaching hospitals in the Netherlands. Patients that were diagnosed with SAB in the period January 2013 to December 2017 were eligible for inclusion. Data on this study cohort have been published previously [7]. All consecutive adult patients (≥ 18 years) with ≥ 1 blood culture positive for *S. aureus* were eligible for inclusion. Patients were excluded if (a) *S. aureus* was detected simultaneously with other pathogens (polymicrobial culture), (b) patients were already on renal dialysis before admission, (c) and AKI occurred prior to the episode of SAB. In patients with multiple episodes of SAB, only the first episode was included. Both patients with community acquired SAB and patients who developed SAB during hospitalization for another indication (hospital acquired SAB) were eligible for inclusion.

Data collection

Blood samples were inoculated in both anaerobic and aerobic bottles and incubated in the BACTEC FX continuous monitoring system (Becton Dickinson BV, Breda, The Netherlands). The clinical data were obtained through review of the electronic patient files. The following data were collected: demographic data, medical history, chronic medication, antibiotic therapy administered for treatment of the SAB episode, vital parameters, and the presence of complicated versus uncomplicated SAB. Baseline serum creatinine (μmol/L), i.e., the most recent known serum creatinine before the presentation with SAB, creatinine at presentation, and maximum creatinine during admission were retrieved from the electronic laboratory system. Furthermore, the time to maximum serum creatinine and the time from maximum creatine to recovery of creatinine were retrieved.

Definitions

Acute kidney injury was defined as 1.5 times baseline creatinine. Recovery of kidney function was defined as creatinine returning to below 1.5 times baseline creatinine during follow-up. The absence of recovery of renal function < 1.5 times baseline creatinine during follow-up was considered non-reversible AKI. Hemodynamic

instability was defined as a mean arterial pressure (MAP) < 65 mmHg or systolic blood pressure < 90 mmHg or need of inotropic or vasopressor agents [8]. Chronic kidney disease was defined as an eGFR < 60 ml/min/1.73 m2. Uncomplicated SAB was defined as an episode of bacteremia with ≥ 1 blood culture with *Staphylococcus aureus*, without evidence of endocarditis/ metastatic infection, and without positive cultures after 48 h of adequate therapy and that was treated for a maximum of 2 weeks, and no relapse occurred, and the patient survived > 72 h after presentation. All situations that did not meet the criteria for uncomplicated SAB were considered complicated SAB. Infective endocarditis was defined by the modified Duke's criteria [9]. Metastatic infection was defined as a clinical and/or radiographical examination and/ or culture concordant with vertebral osteomyelitis, epidural abscess, deep tissue abscess (e.g., psoas) septic pulmonary or cerebral emboli, arthritis, or meningitis.

Statistical analysis

Data were presented as percentages or proportions for categorical variables and as medians plus interquartile range (IQR) for continuous variables. The overall development of AKI and the recovery of AKI were presented as a rate, with 95% confidence interval (95%CI), and were stratified for complicated and uncomplicated SAB. Cox regression analysis was performed to assess time to development and time to recovery of AKI. Recovery of AKI in patients still alive at day 30 was presented as a rate. Univariate analysis was performed by calculating odds ratio's (with 95%CI) and using Fisher's exact tests to identify clinical factors associated with AKI. To assess the correlation of different variables and outcome, a multivariable regression analysis was performed including the variables with $p < 0.20$ from univariate analysis. Subgroup analyses of prevalence of AKI and reversibility were also performed on patients with hemodynamic instability at presentation and patients with preexistent chronic kidney disease.

Ethical approval

Ethical approval was granted by the institutional ethical review committee of the Leiden University Medical Center.

Results

In total, 339 patients with SAB were reviewed. Because of prior chronic (long-term) hemodialysis or development of AKI prior to SAB, respectively 14 and 10 patients were excluded, leaving 315 patients eligible for inclusion in this study. The patient characteristics are summarized in Table 1. In 181/315 (58%) of patients, the SAB

episode met the criteria for complicated SAB. All of the cultured *S. aureus* isolates were methicillin-sensitive (MSSA). Overall 30-day mortality was 21% (67/315).

Table 1. Patient characteristics

	N=315 (100%)
Male sex	213 (67)
Age	68 (57-78)
Comorbidities	
Diabetes	81 (26)
Heart failure	63 (20)
Hypertension	135 (43)
Vascular disease	105 (33)
Chronic kidney disease	53 (17)
Medication	
ACE-i/ARB	103 (33)
Diuretic	101 (32)
Clinical parameters	
Mean arterial pressure	89 (22)
Temperature (°C)	38.5 (37.8-39.1)
Pulse rate (beats/min)	97 (33)
Laboratory parameters	
CRP (mg/L)	148 (68-278)
Leukocytes (x10^9/L)	12.9 (8.6-16.6)
Creatinine (µmol/L)	88 (66-138)
Diagnosis	
Uncomplicated SAB	134 (42)
Complicated SAB	182 (58)
Treatment	
Flucloxacillin	271 (86)
Cephalosporin	21 (7)
Glycopeptide (vancomycin)	10 (3)
Carbapenem	1 (1)
Other	5 (2)
Outcome	
Intensive care	66 (21)
30-day mortality	67 (21)

Legend: Values are count (%) for categorical variables and median (IQR) for continuous variables Chronic kidney disease was defined as an eGFR < 60 ml/min/1,73 m2. Clinical and laboratory parameters are at presentation. Treatment implies the antibiotics prescribed after the first positive blood culture. ACE-i angiotensin-converting-enzyme inhibitors, ARB angiotensin II receptor blockers. Uncomplicated SAB was defined as an episode of bacteremia with ≥ 1 blood culture with *S. aureus*, without evidence of endocarditis/ metastatic infection, and without positive cultures after 48 h of adequate therapy and that was treated for a maximum of 2 weeks, and no relapse occurred, and the patient survived > 72 h after presentation. All situations that did not meet the criteria for uncomplicated SAB were considered complicated SAB.

Incidence and severity of AKI

Acute kidney injury developed in 115/315 (37%; 95%CI 31–42%) of all patients. In the majority of patients, the maximum creatinine was between 1.5 and 2.5 times baseline (Table 2). In patients with complicated SAB, AKI was found more frequently (83/181; 46%) compared to patients with uncomplicated SAB (32/134; 24%; p = < 0.01; OR = 2.70; 95%CI 1.65–4.42). Figure 1a depicts the time from first positive blood culture to maximum creatinine in days, in the 115 patients with AKI. In 45/115 (39%) patients, the maximum creatinine was reached on the day of first blood culture sampling. The median time from first positive blood culture to AKI was 3 days (IQR = 0–11 days). Development of AKI during SAB was associated with 30-day mortality (OR 3.9; 95%CI 2.2–6.9; p < 0.01). In the patients with non-reversible AKI, 27/47 (57%) died within 30 days after blood culture sampling.

Reversibility

Recovery of renal function to < 1.5 times baseline creatinine occurred in 68/115 (59%; 95%CI 49–68%) of patients. There was a small numerical difference in reversibility between complicated and uncomplicated SAB (respectively 60% versus 56%, p = 0.83). The proportion of recovery of AKI was higher in the category of patients with a maximum creatinine of < 2.5 times baseline creatine compared to the more severe kidney injuries (respectively 68% vs 44%, p = 0.02). In patients with reversible AKI, the median time to recovery was two days (IQR = 1–4 days). In 56/68 (82%; 95%CI 73–92%), the recovery occurred within 7 days (Fig. 1b). Among the patients with persistent renal impairment after 7 days, only 12/59 (20%; 95%CI 11–32%) recovered eventually, after temporary renal replacement therapy in five of them. There was no statistically significant difference in reversibility of AKI between patients who presented with AKI and patient who developed AKI during admission (respectively 64% vs 56%, p = 0.45). In the selection of patients still alive at day 30, the recovery rate within 30 days after SAB onset was 52/71 (72%).

Risk factors for AKI

In the univariate analyses, age > 60 years, complicated SAB, chronic kidney disease, cardiovascular disease, the use of diuretics or ACE-i/ARB, hemodynamic instability, temperature > 38.5˚C, and CRP > 150 mg/L, all at baseline, were associated with development of AKI (Table 3). In the multivariable logistic regression analysis, independent riskfactors for AKI were complicated SAB, use of diuretics and hemodynamic instability (Table 3).

Table 2. Gradations of acute kidney injuries

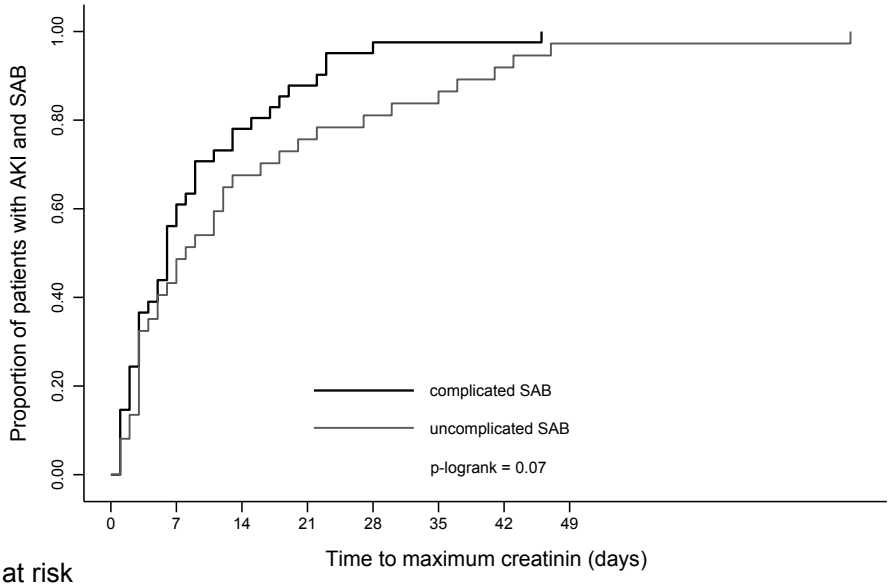
		Total incidence[a]	Recovery of AKI[b]
Maximum creatinine	**1.5x - 2.5x baseline**	74 (64)	51 (68)
	2.5x - 3.5x baseline	17 (15)	10 (59)
	> 3.5x baseline	8 (7)	2 (25)
	Renal replacement therapy	16 (14)	6 (38)

Legend: Total of 115 patients with acute kidney injury (AKI) divided in categories of severity of renal impairment. Values are count (%). [a]Percentages are of column (total group of patients with AKI). [b]Percentages are of row (group of patients in this category of AKI). Recovery of AKI was defined as creatinine drop below 1.5 times baseline creatinine again. Renal replacement therapy was either continuous venovenous hemofiltration (CVVH) or dialysis.

Subgroup analyses

In the subgroup of patients presenting with hemodynamic instability (n = 35), 26/35 (74%) developed AKI. In 12/26 (46%) patients, AKI was reversible. In the subgroup of patients with chronic kidney insufficiency (n = 53), 31/53 (59%) developed AKI. In 16/31 (52%), AKI was reversible.

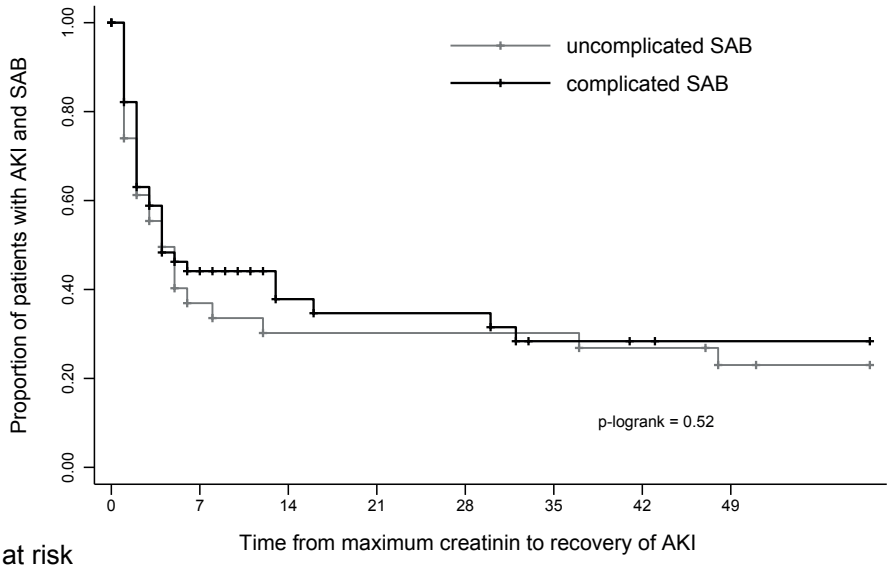
Figure 1. a) Time from blood culture sampling to maximum creatinine in days.



Number at risk

uncomplicated SAB	41	(36)	5	(5)	1	(0)	0	(0)	0
complicated SAB	37	(27)	10	(6)	4	(4)	1	(0)	0

Figure 1. b) Time from maximum creatinine to recovery of creatinine in days.



Number at risk

uncomplicated SAB	50	(28)	9	(1)	8	(1)	5		
complicated SAB	56	(32)	11	(2)	8	(0)	6		

Legend: a Cox regression of all patients with acute kidney injury. Recovery of kidney function was defined as return of creatinine < 1.5 × baseline creatinine. Both: Uncomplicated SAB was defined as an episode of bacteraemia with ≥ 1 blood culture with *Staphylococcus aureus*, without evidence of endocarditis/metastatic infection, and without positive cultures after 48 h of adequate therapy and that was treated for a maximum of 2 weeks, and no relapse occurred, and the patient survived > 72 h after presentation. All situations that did not meet the criteria for uncomplicated SAB were considered complicated SAB

Table 3. Factors associated with development of AKI in SAB

Variable	OR (95%CI)	p-value	B	OR (95%CI)	p-value
Patient characteristics					
Age >60y	1.91 (1.1-3.2)	0.01	0.29	1.33 (0.67-2.63)	0.41
Male sex	0.69 (0.4-1.1)	0.13	-0.50	0.61 (0.33-1.12)	0.11
Complicated SAB	2.73 (1.7-4.5)	<0.01	1.23	3.42 (1.84-6.36)	<0.01
Medical history					
Chronic kidney disease	2.19 (1.3-3.6)	<0.01	0.39	1.06 (0.49-2.30)	0.24
Diabetes	1.45 (0.9-2.4)	0.23			
Cardiovascular disease	2.31 (1.4-3.7)	<0.01	0.06	1.06 (0.49-2.30)	0.87
Malignancy	0.97 (0.6-1.7)	1.00			
Medication					
Use of ACE-i/ARB	1.89 (1.2-3.1)	0.02	-0.31	0.73 (0.35-1.54)	0.41
Use of diuretic agent	3.07 (1.9-5.0)	<0.01	0.70	2.01 (0.99-4.06)	0.05
Clinical and laboratory parameters at presentation					
Hemodynamic instability	6.20 (2.8-13.8)	<0.01	1.97	7.17 (2.51-20.48)	<0.01
Temperature > 38.5˚C	0.59 (0.4-0.9)	0.03	-0.28	0.76 (0.42-1.37)	0.36
Leukocyte count > 15 x10^9/L	1.54 (0.9-2.5)	0.08	0.32	1.37 (0.73-2.57)	0.32
CRP > 150mg/L	1.63 (1.0-2.6)	0.04	0.26	1.30 (0.70-2.39)	0.41

Legend: Univariate and multivariable analysis of risk factors for acute kidney injury in patients with *S. aureus* bacteremia. OR odds ratio, B regression coefficients. Chronic kidney disease was defined as an eGFR < 60 ml/min/1,73 m2. Cardiovascular disease consists of hypertension, vascular disease, and/or heart failure. Hemodynamic instability was defined as a mean arterial pressure (MAP) < 65 mmHg or systolic blood pressure < 90 mmHg or need of inotropic or vasopressor agents. ACE-i angiotensin-convertingenzyme inhibitors, ARB angiotensin II receptor blockers, CRP C-reactive protein.

Discussion

The main finding of our study is the high overall incidence of AKI in patients with SAB (37%), particularly in patients with complicated disease. This high incidence, combined with the limited reversibility, illustrates the significance of this complication.

We found that AKI in SAB develops early in most patients. In a high proportion (39%) of patients developing AKI the creatinine level peaked at the day of first positive blood culture. Furthermore, the median time to peak creatinine was 3 days after first positive blood culture. These findings are similar with those reported by Holmes et al. [3]. The slightly higher incidence of AKI in the study by Holmes may be explained by a different definition of AKI. They included low urine output in their definition, whereas our definition was based on serum creatinine alone. Other research on AKI in SAB is limited to studies that were primarily aimed at comparing treatment outcome of different antibiotic therapies. In these studies, the incidence of nephrotoxicity was highly variable, ranging from 2 to 33% [10–14].

Acute kidney injury was reversible in the majority of patients (59%), but a significant proportion of patients suffered from irreversible renal impairment. In patients with reversible AKI, recovery occurred within 7 days after onset in the majority of patients (82%). Persistent kidney injury beyond this time point is prognostically unfavorable. In patients with persistent AKI at T = 7 days, recovery was observed in only 20%. The high proportion of non-reversible AKI in our study may partially be explained by disease severity. The association between disease severity and both the prevalence and the reversibility of AKI has been demonstrated for sepsis-associated kidney injury in general [15,16]. Several risk factors for the development of AKI were identified in our study. Apart from diagnosis of complicated SAB, the use of diuretics as well as hemodynamic instability at time of admission remained independent risk factors for AKI in multivariable analysis. Together with the time course of renal insufficiency showing early onset and quick recovery, this finding suggests that hemodynamic deterioration early in the disease plays an important role in the development of AKI.

However, the results of our study do not yield definite answers regarding pathophysiology. Toxicity of antibiotics, i.e., nafcillin and aminoglycosides, has been suggested in the literature to be important in development of AKI, although this assumption was not confirmed by kidney biopsies [10–14, 17, 18]. In the current study, the vast majority (86%) of patients was treated with flucloxacillin according to the Dutch guideline, limiting the comparison of different antibiotic therapies on AKI development [19, 20]. However, based on the median time to AKI of 3 days, toxicity caused by antibiotic therapy does not seem to have been a major cause of AKI. For example, TIN on antibiotic therapy is unlikely if the onset is < 5 days after start of antibiotic therapy [21]. Secondly, TIN is unlikely to recover within 1 week.

This is relevant, as falsely attributing AKI to beta-lactams may deter a patient from optimal antibiotic treatment.

The current lack of non-invasive diagnostic tools to differentiate between the divergent etiologies of AKI in SAB leads to misdiagnoses that cannot be refuted. Insight in the etiology of AKI in SAB and the probability of different causal mechanisms has important diagnostic and therapeutic consequences and warrants prospective studies, focusing on etiology. Urine biomarkers could possibly be of additional value herein, but still need future research.

An association between occurrence of AKI and 30-day mortality in patients with SAB was previously reported and confirmed in this study [22]. Although causality cannot be determined based on either study, AKI is likely to affect patient outcome on theoretical grounds. Patients with AKI— in general—are at increased future risk of chronic kidney disease and death [23]. The high burden of morbidity and mortality stresses the importance of further studies on AKI in SAB.

An important limitation of our study is the fact that the cause of AKI was rarely proven histologically, limiting insights in the etiology of SAB in our population. The lack of biopsy-confirmed etiologic diagnoses in both our study and previously mentioned studies is a reflection of daily practice, as renal biopsies are rarely performed [10–14, 17, 18]. A second limitation of this study is the retrospective design. Variables that were not measured—such as aminoglycoside therapy— may be associated with the development of AKI in SAB.

In conclusion, this study shows that AKI is common in patients with SAB. The risk factors found, and the swift reversibility in most patients, suggest that a major cause for AKI is hemodynamic in nature. This knowledge may provide insights that support diagnostic and therapeutic management of patients with SAB. Future prospective intervention studies are warranted to evaluate the underlying pathophysiology and potential interventions.

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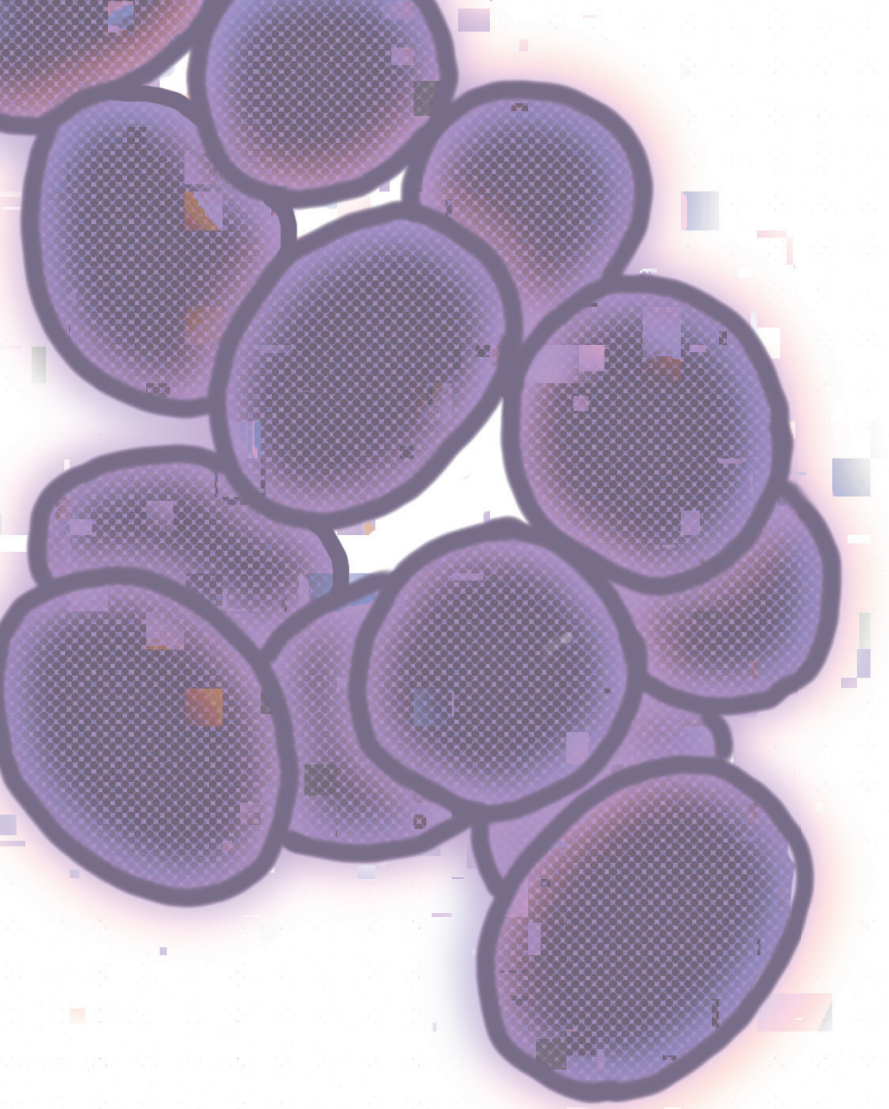

Chapter 8

Persistent MRSA bacteremia: host, pathogen and treatment

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a devastating pathogen responsible for a variety of life-threatening infections. A distinctive characteristic of this pathogen is its ability to persist in the bloodstream for several days despite seemingly appropriate antibiotics. Persistent MRSA bacteremia is common and is associated with poor clinical outcomes. The etiology of persistent MRSA bacteremia is a result of the complex interplay between the host, the pathogen, and the antibiotic used to treat the infection. In this review, we explore the factors related to each component of the host–pathogen interaction and discuss the clinical relevance of each element. Next, we discuss the treatment options and diagnostic approaches for the management of persistent MRSA bacteremia.

Introduction

With almost 20,000 deaths attributed to *Staphylococcus aureus* bloodstream infections in the USA in 2017, *S. aureus* bacteremia (SAB) is one of the most frequent and severe bacterial infections [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common cause of infections due to multidrug-resistant bacteria in the United States [2]. Bacteremia due to MRSA has long been associated with higher mortality rates than its more susceptible counterpart [3]. Although most studies have shown higher mortality rates, MRSA bacteremia (MRSAB) has only a slightly higher adjusted mortality compared to methicillin-susceptible SAB [4]. More recent high-quality studies in the field suggest a limited odds ratio (OR) or relative risk (RR) increase in death of around 1.3–1.8 [4].

We have learned over the decades that mortality in patients with SAB can be decreased through standardized clinical management practices such as obligatory infectious diseases consultation, routine echocardiography and follow-up blood cultures, and appropriate antibiotics [5–10]. Despite these insights, ≈25% of patients with SAB will die within 3 months of diagnosis [4].

One of the unique and disturbing features of SAB is the tendency of the organism to persist in the bloodstream despite the presence of microbiologically appropriate antibiotics. The phenomenon of persistent bacteremia remains poorly understood, and we lack great tools to identify who is at risk for persistent SAB.

This paper reviews the basic science and clinical literature behind persistent MRSAB. We discuss the contribution from the host and the pathogen in the pathophysiology of SAB.

Persistent MRSAB

Persistent SAB is the strongest predictor of complicated SAB [11]. Multiple observational studies have identified the stark difference in mortality in patients with persistent SAB compared to those whose bacteremia promptly resolves [12–14]. One recent cohort of 884 patients with SAB (approximately one-third with MRSAB) determined that increasing duration of positive *S. aureus* blood cultures was associated with increased rates of metastatic complications, length of stay, and 30-day mortality [12]. The investigators concluded that each additional day of bacteremia was associated with a relative risk of death of 1.16 [12]. Another multinational cohort of 1588 patients with SAB found that 90-day mortality almost doubled (22 to 39%) when the duration of bacteremia increased from 1 day to 2–4 days [14]. Both studies underlined the severe consequences of persistent SAB. The consequences relating to treatment and further diagnostic evaluation are discussed later in this review.

Both the definition and the frequency of persistent SAB have evolved over the past two decades [15]. In the early 2000s, Fowler et al. defined persistent bacteremia as ≥7 days of positive blood cultures [16] on the basis of the median duration bacteremia in patients with MRSA [17,18]. The reliable therapeutic options for MRSAB during that era were limited to vancomycin only. As a result, the designation of persistent MRSAB had little therapeutic consequence, as in most clinical cases, the vancomycin was simply continued. Since then, however, several new antibiotics with effectiveness against MRSA have been approved by the Food and Drug Administration (FDA). One antibiotic, daptomycin [19], has been approved specifically for MRSAB. In addition, other antibiotics such as the fifth-generation cephalosporin ceftaroline [20] are frequently used off-label for MRSAB. Given the ability to use alternate antibiotics and some data supporting combination antibiotic therapy for MRSAB (discussed in Section 4.2), more recent reports have suggested modifying the definition of persistent MRSAB to include patients with positive blood cultures for as few as 2 days [14].This shorter duration allows for a “check point” to consider alternate therapy and broader diagnostic evaluation [21].

Host factors associated with persistent MRSAB

Clinical risk factors

Numerous observational studies have identified independent patient risk factors for the development of persistent SAB (Table 1) [22–28]. A recurring theme is the presence of retained intravascular devices or foreign bodies, which are independently associated with persistent SAB [15,22,24–26,28]. Similarly, metastatic infection (including endocarditis, bone and joint infection), chronic renal failure, cirrhosis, and diabetes are also associated with persistent SAB [22,23,25,26,28]. The largest study was a nested case–control study examining risk factors for persistent SAB, performed by Chong et al., who included 483 patients with persistent SAB and 212 patients with resolving SAB [22]. In addition to the previously described risk factors, multivariate analysis revealed community-onset bacteremia, methicillin resistance, central venous catheter (CVC)-related infection, and vancomycin trough of <15 mg/L as risk factors for persistent SAB [22].

The majority of these studies do not distinguish methicillin-susceptible *S. aureus* (MSSA) from MRSAB, often citing vancomycin use as a risk factor for persistence [23,26]. Yoon et al. limited their investigation to MRSA only, identifying retention of implanted devices and metastatic infection of at least two sites as predictors of persistent MRSAB [24]. While these studies represent an important component in the understanding of persistent SAB and MRSAB, it currently comes as little surprise that unresolved sources of infection are the most frequently reported clinical risk factors for persistence. However, clinical risk factors only partially explain which patients develop persistent SAB.

Table 1. Clinical risk factors for persistent SAB.

Study	Year	MSSA or MRSA	Definition of Persistence	Clinical Risk Factors Identified
Khatib et al. [28]	2006	MSSA and MRSA	3 days	• Intravascular catheter (RR, 1.27; 95% CI 1.03–1.54) • Cardiovascular prosthesis (RR, 1.24; 95% CI 0.97–1.59) • Metastatic infection (RR, 1.16; 95% CI 1.05–1.28)
Hawkins et al. [27]	2007	MSSA and MRSA	7 days	• Chronic renal failure (OR, 2.08; 95% CI 1.09–3.96) • >2 sites of infection (OR, 3.31; 95% CI 1.17–9.38) • Infective endocarditis (OR, 10.3; 95% CI 2.98–35.64) • Presence of intravascular catheter or foreign device (OR, 2.37; 95% CI 1.11–3.96)
Khatib et al. [25]	2009	MSSA and MRSA	7 days	• Metastatic infection (OR, 5.6; 95% CI 3.00–10.47) • Vancomycin treatment (OR, 4.17; 95% CI 2.14–8.11) • Endovascular source (OR. 3.35; 95% CI 1.92–5.85) • Diabetes (OR, 2.14; 95% CI 1.26–3.64)
Ganga et al. [30]	2009	MRSA and MSSA	7 days	• Metastatic infection (OR, 11.35; 95% CI 4.24–31.43 • Diabetes (OR, 3.64; 95% CI 1.45–9.155) • Prosthetic device (OR, 3.22; 1.30–8.00)
Yoon et al. [26]	2010	MRSA	7 days	• Retention of infected medical device (OR, 10.35; 95% CI 1.03–104.55) • Infection of at least two metastatic sites (OR, 10.24; 95% CI 1.72–61.01)
Chong et al. [24]	2013	MSSA and MRSA	7 days	• Community-onset bacteremia (OR, 2.91; 95% CI, 1.24–6.87) • Bone and joint infection (OR, 5.26; 95% CI, 1.45–19.03) • Central-venous-catheter-related infection (OR, 3.36; 95% CI, 1.47–7.65) • Metastatic infection (OR, 36.22; 95% CI, 12.71–103.23) • Delay in removal of eradicable foci >3 days (OR, 2.18; 95% CI, 1.05–4.55)

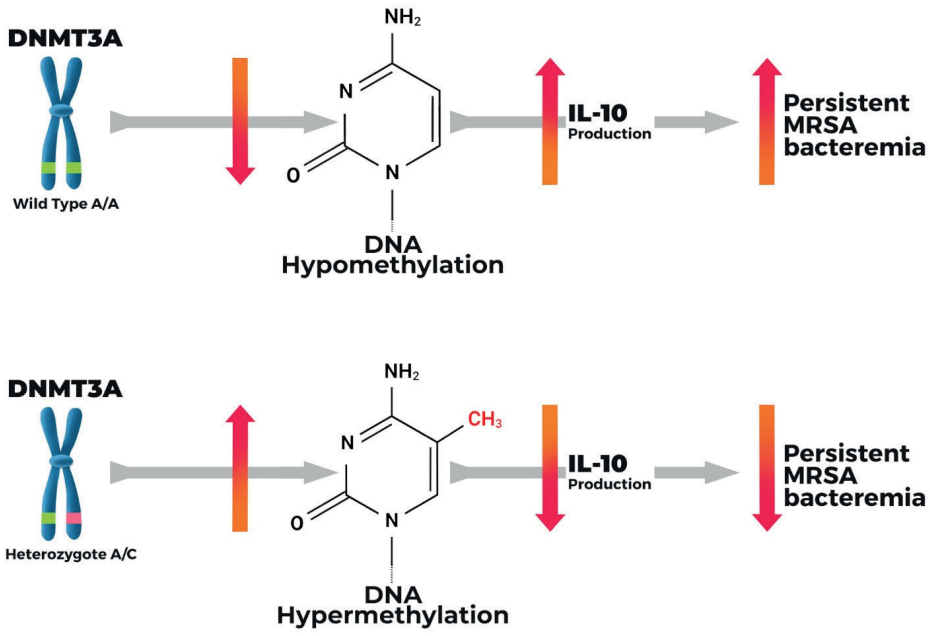
Abbreviations: MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; RR, risk ratio; CI, confidence interval; OR, odds ratio.

Host genetic variation and SAB

Genetic risk factors for infection have been identified in a wide range of infectious diseases [29]. A landmark study performed in the 1980s determined children of adults who experienced premature death due to infection were more likely to experience death due to infection themselves, suggesting a heritable basis for their infection risk [30]. Rare primary immunodeficiency syndromes such as chronic granulomatous disease, hyper-IgE syndrome, and Chédiak–Higashi have been associated with increased susceptibility to *S. aureus* infection [31–34]. Few studies have examined the genetic risk factors for *S. aureus* bloodstream infections and even less focus on persistent MRSAB. A fascinating study by Oestergaard et al. was performed in 2016 by examining a database consisting of almost all parents and children born in Denmark between 1954 and 2016 (n = 8,951,393) [35]. On the basis of 18,626 reported cases of SAB and 34,774 first-degree relatives, the investigators found that first-degree relatives of patients hospitalized for SAB were more likely to experience an episode of SAB themselves (standardized incidence ratio (SIR) of 2.49; 95% confidence interval (CI) 1.95–3.19). The risk was particularly notable in siblings of patients with SAB (SIR, 5.01; 95% CI 3.30–7.62) compared to parents (SIR, 1.96; 95% CI 1.45–2.67). While these data provide compelling evidence for heritable risk factors for acquiring SAB, the specific genetic defect remains unknown.

Three genome-wide association studies (GWAS) have been performed to identify host genetic variability that can predispose to SAB. Two smaller studies by Nelson et al. (361 SAB cases and 699 controls) and Ye et al. (309 cases and 2925 controls) did not identify single-nucleotide polymorphisms (SNPs) with genome-wide significance for risk of acquiring or severity of SAB [36,37]. A third larger GWAS study of 4701 SAB cases and 45,344 matched controls identified two SNPs that achieved genome-wide significance for altered susceptibility to *S. aureus* infection in individuals of European ancestry (rs35079132: $p = 3.8 \times 10^{-8}$, and rs35079132 $p = 3.8 \times 10^{-8}$) [38]. These loci were located near the HLA-DRA and HLA-DRB1 genes in the HLA class II region. Using admixture mapping, that same genetic region of European origin was also identified in African Americans as associated with SAB at a genome-wide level of significance [39]. This discovery was the first of its kind in *S. aureus* research and built on the enlarging body of evidence linking HLA haplotypes to susceptibility and severity of bacterial infection [40–45].

Figure 1. Proposed relationship of DNMT3A polymorphisms and increased risk of persistent MRSAB. Created using Biorender.



Host genetic variation and persistent MRSAB

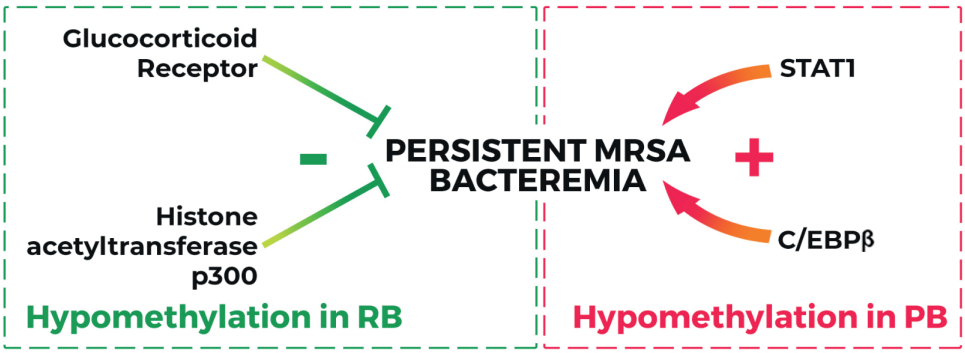
Despite the advances in our understanding of genetic risk factors for SAB, none of these studies addressed which genetic variants protect or place patients at risk of persistent methicillin-susceptible or methicillin-resistant SAB. A breakthrough discovery was made by Mba Medie et al., who identified a key association between genetic variation in the DNMT3A gene and protection against persistent MRSAB [46]. This elegant study performed whole-exome sequencing (WES) on a cohort of 68 patients with persistent MRSAB (n = 34), defined as persistently positive blood cultures for ≥5 days, and resolving MRSAB (n = 34), defined as blood culture positivity for <5 days. These patients were matched by sex, age, race, presence of implanted devices, diabetes mellitus status, and hemodialysis status. The study revealed a specific polymorphism (g.25498283A > C) in the DNA methyltransferase 3A intronic region of DNMT3A that was associated with a reduced risk of persistent MRSAB. The variant was identified in 61.8% of the cohort with resolving bacteremia and just 8.8% of patients with persistent bacteremia ($p = 7.8 \times 10^{-6}$). Examination of the DNA methylation patterns between patients with and without the g.25498283A > C

mutation revealed significantly higher levels of methylation in gene-regulatory CpG island regions in patients expressing the homozygous genotype. Cytokine analysis also revealed significantly lower levels of anti-inflammatory cytokine interleukin-10 (IL-10) in acute phase serum from patients with resolving MRSAB compared to persistent MRSAB (114 pg/mL in persistent bacteremia patients vs. 13.1 pg/mL in resolving bacteremia patients; p = 0.009). IL-10 levels were also found to be lower in the subset of patients with the g.25498283A > C polymorphism, regardless of whether the serum was from patients with persistent MRSAB or resolving MRSAB (A/C: 18.9 pg/mL vs. A/A: 68.9 pg/mL in patients with persistent MRSAB and A/C:8.7 pg/mL vs. A/A:14.95 pg/mL in patients with resolving MRSAB). The proposed mechanism for decreased susceptibility to persistent MRSAB is thought to revolve around suppression of IL-10 production via DNA-methyltransferase-3A-mediated DNA methylation (Figure 1). While the exact role of IL-10 in promoting persistent MRSAB is unclear, this finding was consistent with prior studies that also found an association between elevated IL-10 and mortality from SAB and persistent SAB [13,47]. IL-10 is an immunosuppressive cytokine and is known to prevent the activation of Th1 helper T cells and subsequently can increase survival of some intracellular bacteria [48]. It is known that IL-10 signaling can suppress proinflammatory macrophage and cytokine production, resulting in less reactive oxygen species (ROS) and reactive nitrogen species (RNS) known to play a crucial role in fighting *S. aureus* and other pathogens [48–52]. One can hypothesize that the reduced IL-10 production in patients with the g.25498283A > C polymorphism allows for a more robust pro-inflammatory response, which assists with efficient clearance of bacteria from the bloodstream. However, more research in this field is needed to further unravel the complex mechanism.

A 2020 follow-up study by Chang et al. examined the DNA methylation pattern in leukocytes from 142 patients with persistent MRSAB (blood culture positive >5 days; n = 70) and resolving MRSAB (blood culture positive <5 days; n = 72) [53]. This study used advanced sequencing techniques to quantify and localize differences in the DNA methylome. DNA extracted from persistent MRSAB patients' leukocytes exhibited significantly lower levels of methylation localized to binding sites for two transcription factors involved in immune regulation: signal transducer/activator of transcription 1 (STAT1) and CCAAT enhancer binding protein-β (C/EBPβ) (Figure 2). In contrast, the profile of the resolving MRSAB patients' methylome localized differences in the histone acetyltransferase p300 and glucocorticoid receptor binding site. The mechanistic basis for these changes is proposed by the authors. Firstly, C/EBPβ has a role in emergency granulopoiesis [54], and the abundance of immature granulocytes arising from activation of the C/EBPβ gene may impair the ability of the immune system to assimilate the circulating bacteria, promoting persistence.

Second, activation of STAT1 is known to induce T-helper cell polarization into the Th1, which tips the see-saw balance away from Th17-mediated interleukin-1 (IL-1) and interleukin 17 (IL-17) production known to mediate neutrophil recruitment and activation critical for bactericidal activity. Third, in resolving persistent MRSAB patients, the hypomethylation in glucocorticoid receptor and associated co-factor p300 histone acetyltransferase promoter regions likely helps counter-regulate the life-threatening pro-inflammatory response that occurs during bloodstream infections [55].

Figure 2. Schematic showing genes with hypomethylation in patients with persistent MRSA bacteremia (PB) and resolving MRSA bacteremia (RB).



Biomarkers for persistent SAB

These studies represent a potential breakthrough in unraveling the astonishingly complex genomic and epigenetic distinctions between patients with persistent MRSAB and resolving MRSAB. The clearest application of this discovery is the potential to identify patients at risk for persistent MRSAB, which could lead to alterations of initial therapy, expediting of additional diagnostic evaluation, and the capacity to improve clinical outcomes. Concurrent work in identifying biomarkers in patients with persistent SAB and persistent MRSAB has identified a handful of possible candidates. Using a threshold of blood cultures positive for >5 days to define persistent SAB, Guimaraes et al. identified eight proteins correlating with persistent SAB, with interleukin 17A (IL-17A), IL-10, and soluble E-selectin levels, showing the most robust association [47]. A follow-up study by Cao et al. found levels of IL-17A, IL-10, or soluble E-selectin levels were able to individually identify patients at risk of

microbiologic failure and persistent SAB [56]. These biomarkers were more predictive than clinical risk factors known to increase risk for persistence (age, steroid use, hemodialysis, non-removable infection foci, hospital vs. community onset, and MRSA vs. MSSA). Given the association of persistent SAB with mortality, it is unsurprising that elevated IL-17A and IL-10 levels were each associated with increased mortality in this study [13,56].

While these discoveries are exciting and show promise for future diagnostic options to stratify patient risk for persistence, the clinical utility at the present day is hampered by availability only in specific academic centers and reliance on external laboratories to perform the tests. Fast turnaround time will be the key to the real-world use of these tests to identify patients at risk of persistent SAB. This could allow for early detection of persistent SAB and subsequently altered therapeutic and diagnostic strategies that could potentially save lives.

Pathogen-associated risk factors for persistent *S. aureus* bacteremia

To survive and replicate in the bloodstream, *S. aureus* must avoid a barrage of host defenses while attempting to adhere to and proliferate upon an endothelial surface of the vasculature. The establishment of endovascular infection is a complex process requiring coordinated expression of multiple adhesins, exotoxins, and exoenzymes at various stages of infection. Meanwhile, *S. aureus* must resist or avoid phagocytosis by neutrophils and the resulting oxidative and non-oxidative burst, in addition to the circulating platelet-derived antimicrobial peptides. There is significant heterogeneity in the catalog of virulence factors produced by different *S. aureus* clinical isolates [57–60], the regulators mediating virulence factor expression [61–64], and susceptibility to antimicrobial peptides [65–68]. This section discusses the key genetic and phenotypic characteristics of *S. aureus* that have been associated with persistent SAB.

Accessory gene regulator dysfunction

Virulence factor production is tightly controlled by a series of regulatory mechanisms including several two-component systems and SarA-family regulators [69]. One of the most well-characterized global regulators of virulence factor production is the two-component quorum-sensing accessory gene regulator (agr) system of *S. aureus* [70]. The agr system is a quorum-sensing system that mediates expression of exotoxins and exoenzymes [69]. The essentiality of agr to virulence in *S. aureus* infection depends on the type of infection [70]. Murine skin and soft tissue models have shown that agr deletion mutations are severely attenuated. However, agr-null *S.*

aureus strains are frequently isolated from the bloodstream of human subjects with SAB [16,61–63,71–73]. Several groups have shown that specific agr genotypes are associated with persistent MRSAB [16,74,75]. Fowler et al. discovered that isolates from patients with persistent MRSAB were predominantly (≈85%) of similar agr genotypes and lacked agr activity, as measured by δ-lysin production. The same study also noted that isolates from patients with persistent MRSAB were less susceptible to killing by thrombin-induced platelet microbicidal protein, an antimicrobial peptide produced by host platelets. Another study by Park et al. examined the agr genotype in MRSAB patients without retained foci of infection (e.g., prosthetic joint, intravenous catheter) [74]. They found that persistent MRSAB isolates more frequently possessed agr dysfunction compared to those from patients with resolving bacteremia (94% vs. 75%, p = 0.03). A third investigation by Kang et al. limited their investigation to 152 patients with persistent MRSAB and asked if infections due to isolates with agr dysfunction had worse clinical outcomes compared to agr positive strains [75]. They found significantly higher rates of in-hospital mortality in patients with persistent MRSAB if the bloodstream isolate had a dysfunctional agr system (68% vs. 49%, p = 0.029). The mechanism for the reciprocal relationship between agr activity and persistence remains unclear but is likely multifactorial. First, the reduction in cytotoxic leukocidin production in agr-null isolates may lead to decreased host-cell toxicity and increased bacterial survival [75]. Second, the agr operon also repressed adhesins such as fnbA, which are required for adhesion and invasion of endothelial cells. The lack of a functional agr would result in upregulated adhesins and potentially enhanced intracellular invasion, where it would be shielded from the effects of numerous antibiotics including vancomycin. Third, multiple studies have linked agr dysfunction with glycopeptide intermediate-resistance or vancomycin tolerance (discussed further in Section 3.4 The mechanism of increased antibiotic tolerance is thought to be due to altered autolysin activity, blunting the bactericidal effect of vancomycin [61,74]. These studies provide some compelling evidence that agr dysfunction can be a driver of persistent SAB.

Variability in virulence factor production

Despite several decades of mechanistic studies examining *S. aureus* virulence factor function and regulation, the field has been unable to pinpoint which specific virulence factors are responsible for microbial survival in bloodstream infections. It appears that no single virulence factor can dictate the pathophysiology, which points towards combinations that are likely expressed in different infectious niches. Few studies have examined virulence factor expression to specifically differentiate persistent MRSAB from resolving MRSAB isolates. Xiong et al. performed an in vitro analysis on isolates from patients with persistent MRSAB and resolving MRSAB to determine phenotypic characteristics that may distinguish the two isolates [76]. They found that

isolates from persistent MRSAB patients differed in several characteristics. First, the persistent MRSAB isolates were more resistant to killing by hNP-1, an antimicrobial peptide produced by neutrophils. Second, they discovered that persistent MRSAB isolates were more adept at binding to fibrinogen and fibronectin, which are thought to act as the anchors allowing *S. aureus* to establish endovascular infection. Third, multiplex genotyping identified the genes cna, sdrD, and sfrE more frequently in persistent MRSAB isolates compared to resolving MRSAB isolates. However, another larger study using the same definition of persistent MRSAB (cultures positive >7 days) was unable to find differences in the presence of virulence factor genes (including sdrD) or agr dysfunction [22]. Similarly, Seidl et al. did not note any differences in fibronectin binding between persistent versus resolving MRSAB isolates [77]. These inconsistencies between studies may highlight epidemiological differences between SAB isolates from different geographic centers.

Phenotypic variability of SAB isolates

While genotypic analysis has been extremely informative in differentiating persistent MRSAB from resolving MRSAB isolates, often the downstream effects on function are a result of multiple interacting processes. Following on from Xiong et al.'s work discussed in Section 3.2, Seidl et al. performed several in vitro studies to distinguish functional differences between isolates from patients with persistent MRSAB vs. resolving MRSAB [77]. They again confirmed that persistent MRSAB isolates exhibited significantly less killing by the neutrophil-derived AMP hNP-1 ($p = 0.02$) and platelet-derived thrombin-induced platelet microbicidal proteins (tPMPs, $p = <0.001$). Other findings from the study noted no significant difference in overall biofilm biomass produced, but they did report biofilms from persistent MRSAB isolates contained a lower carbohydrate content (58.4% vs. 30.6%; $p = 0.04$). It is thought that platelet-derived antimicrobial peptides, such as tPMPs, play a key role in assisting clearance of *S. aureus* in the bloodstream, particularly around areas of endothelial damage that are thought to serve as an anchor in the establishment of an endovascular infection [78]. *S. aureus* isolates exhibiting decreased killing by tPMPs in-vitro show increased virulence in an in vivo rabbit endocarditis model [66,79]. Furthermore, *S. aureus* bloodstream isolates from patients with confirmed endovascular infections were less susceptible than bacteremia strains without an endovascular source [67,68]. It is reassuring to see the clinical relevance of the in vitro studies by establishing the relationship between decreased tPMPs killing and persistent MRSAB [16,76]. The relationship between decreased hNP-1 killing and persistence is less well established but could be a result of increased survival inside neutrophils after phagocytosis [76,77].

Antibiotic tolerance

Antibiotic resistance is the inherited ability of bacteria to grow in the presence of elevated concentrations of antibiotics and is quantified by measuring the minimum inhibitory concentration (MIC). Antibiotic tolerance refers to the ability of a population of bacterial cells to survive in the presence of lethal concentrations of bactericidal antibiotics without a change in the MIC [80]. Resistance generally involves a specific mechanism, such as modification of the target, efflux pumps, or deactivation of the antibiotic, whereas the mechanisms of antibiotic tolerance are more general and are commonly associated with slower growth and decreased metabolic activity. The absence of MIC alteration and the wide variability in the pathways that lead to tolerance means the phenotype is challenging to detect. There is currently no standardized testing protocol allowing for detection of antibiotic tolerance in the clinical microbiology laboratory. Additionally, tolerance is highly dependent on the environment, making it difficult to measure under ex vivo conditions. Studies have shown a proportion of *S. aureus* can survive phagocytosis by host immune cells and persist in the intracellular space [81]. Due the poor intracellular permeability of antibiotics such as vancomycin and daptomycin, these intracellular bacteria are shielded from the effects of serum antibiotics [82]. Recent work by Rowe et al. discovered that host immune cells can also induce antibiotic tolerance in *S. aureus* by ROS-mediated inactivation of key tricarboxylic acid cycle (TCA) enzymes [83,84]. Another mechanism of host-induced tolerance was identified by Ledger et al., who report that human serum can induce daptomycin tolerance through LL-37-mediated activation of the GraRS two-component system and membrane lipid remodeling [85]. These studies emphasize the diversity in the mechanisms of antibiotic tolerance and underline the difficulty of detecting these phenotypes once the bacteria is removed from the host environment. The most common method for determining antibiotic tolerance is by performing a time-kill curve, which looks at the rate of antibiotic killing of a pathogen by an antibiotic over time [86], which is laborious and not feasible in a busy clinical microbiology laboratory. The devastating consequences of antibiotic resistance are ubiquitously acknowledged through the scientific community, although the clinical impact of antibiotic tolerance is less well understood. In addition, there is no standardized definition of antibiotic tolerance, although some groups have agreed that a minimum bactericidal concentration (MBC) to MIC ratio of >32 is consistent with tolerant bacteria [87–90]. A key study by Levin-Reisman revealed that antibiotic tolerance acts as a precursor to antibiotic resistance [91]. The mechanism proposes that decreased antibiotic killing in antibiotic-tolerant cells results in an increase in the pool of viable cells available to acquire mutations that confer resistance. Further studies are needed to explore if this phenomenon can be extrapolated beyond ampicillin tolerance and resistance in *Escherichia coli*. While the clinical relevance of this finding will require further experiments, it provides further

evidence that tolerance may be an unappreciated pathway to treatment failure [91].

Glycopeptide tolerance has been frequently observed in *S. aureus*, with a prevalence of up to 43% in MRSA isolates [87,92]. While it is suspected that antibiotic tolerance is a contributor to refractory and relapsing infections, there are few studies that have directly addressed this question. Given the definition of decreased antibiotic killing in antibiotic tolerance, one could hypothesize that antibiotic tolerance may play a role in persistent bacteremia. Britt et al. performed a retrospective cohort study of 225 patients with SAB comparing frequency of clinical failure (30 day all-cause mortality, persistent signs and symptoms of bacteremia, recurrent bacteremia within 30 days, and blood culture positive >5 days) between isolates with and without vancomycin tolerance [88]. In their study, 26.7% of the isolates exhibited vancomycin tolerance, which was associated with clinical failure in unadjusted (68.3% vs. 40.6%) and multivariable analysis (adjusted risk ratio, 1.74; 95% CI, 1.35–2.24; $p < 0.001$). The average bacteremia duration did not significantly vary between the two groups, nor did the proportion with blood cultures positive for >3 days (48.2% in vancomycin-tolerant (VT) vs. 38.4% in non-VT). Another smaller study of 163 patients with MRSAB from St. Louis, USA, noted just 4.3% of isolates were vancomycin-tolerant with no statistically significant effect on clinical outcomes. Finally, a study by Moise et al. noted increased duration of bacteremia (median time to clearance 6.5 days vs. >10.5 days, $p = 0.025$) when MRSA isolates were stratified by tolerance (≤2.5 log10 decrease in colony-forming units/mL over 24 h of vancomycin treatment) [93]. Larger studies are needed to determine the clinical impact of antibiotic tolerance in persistent MRSAB.

The mechanisms of antibiotic tolerance are incompletely understood, especially in *S. aureus*. To identify if antibiotic tolerance evolves within patients, Elgrail et al. performed WGS on 206 MRSA isolates from 20 patients with persistent MRSAB [94]. Their results showed that MRSA can evolve antibiotic tolerance within the host due to mutations in the TCA cycle (odhA and citZ) and stringent response (relA). Interestingly, these mutants were transient and were not present in subsequent positive blood cultures, suggesting there is phenotypic heterogeneity and a fitness cost to tolerance, which has been described in other pathogens [95].

Reduced vancomycin susceptibility and heterogenous vancomycin-intermediate *S. aureus*

Vancomycin is the oldest and most frequently used drug in our arsenal against MRSA [96]. Despite being used for almost 65 years, vancomycin resistance (MIC ≥ 16 µg/mL) is extraordinarily uncommon, with just 52 incidents of vancomycin-resistant *S. aureus* (VRSA) reported worldwide in the past two decades [97]. Vancomycin-intermediate *S. aureus* (VISA) is defined by a vancomycin MIC between 4 and 8 µg/mL

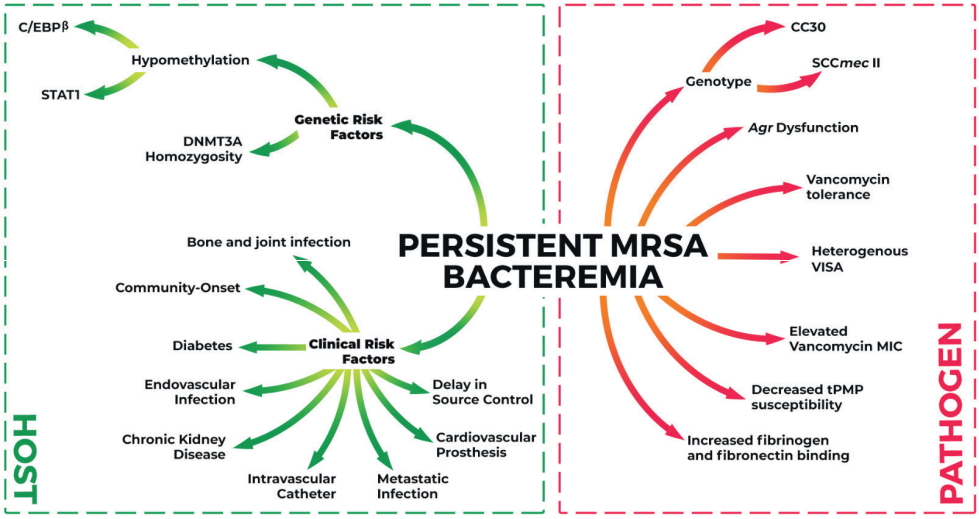
and is more frequent with an estimated prevalence of between 0.3 and 18% depending on the geographic area [98]. In theory, vancomycin is an appropriate treatment for MRSAB isolates with vancomycin MIC between 1 and 2 μg/mL. There has been a long-standing debate questioning whether MRSA with elevated vancomycin MIC (>1.5 μg/mL) is associated with worse clinical outcomes or not. The majority of data, including two systematic reviews and meta-analyses, indicates that MRSAB due to isolates with high vancomycin MIC (>1.5 μg/mL) is associated with increased mortality compared to MRSAB due to isolates with low-vancomycin MIC (<1.5 μg/mL) [93,99,100]. This finding is not necessarily related to failure of vancomycin, as an elegant study by Holmes et al. also found worse clinical outcomes in MSSA bacteremia isolates with elevated vancomycin MIC, despite treatment with flucloxacillin and not vancomycin [101]. This finding is consistent with the Infectious Disease Society of America (IDSA) recommendations to base treatment decisions in patients infected with MRSA isolates with vancomycin MIC of 2 μg/mL upon clinical conditions [91]. The majority of studies examining the risk of elevated vancomycin MIC with clinical outcomes used composite outcomes for treatment failure, often including (but not always specifying) persistent bacteremia [100]. When the systematic review and meta-analysis by van Hal et al. limited their analysis exclusively to studies that examined persistent MRSAB, the OR was 2.44 but was not significant (95% CI, 0.72–8.24) [100]. Some individual studies did show an association, such as a retrospective cohort of 222 MRSAB patients by Neuner et al. that identified a significantly higher rate of persistent MRSAB when vancomycin MIC was 2 μg/mL compared to <2 μg/mL (16% vs. 5 %, p = 0.012) [102]. Another smaller study by Yoon et al. also found vancomycin MIC of 2 μg/mL is an independent predictor of persistent MRSAB (OR 6.34; 95% CI, 1.21–33.09) [65]. Another newer study by Adani et al. of 166 patients from an institution with blinded vancomycin MIC showed no significant difference in persistent bacteremia rates between isolates with MIC < 2 μg/mL vs. 2 μg/mL (16.5% vs. 17.3%, p = 0.884) [103].

Heterogenous VISA (hVISA) is another microbiologic phenomenon that could contribute to decreased vancomycin efficacy [104]. The first reported case of hVISA was in 1996 from a patient in Japan with MRSA pneumonia that did not respond to vancomycin [105]. Despite susceptibility testing showing vancomycin MIC of 4 μg/mL, a subpopulation was discovered with MICs ranging from 5 to 9 μg/mL. An isolate with vancomycin MIC in the susceptible range (≤2 μg/mL) with a subpopulation with vancomycin MIC in the intermediate range (4–8 μg/mL) has become diagnostic of hVISA [106]. Similar to the challenges of identifying antibiotic tolerance, the detection of hVISA is laborious and utilizes the population analysis profile (PAP) area under the curve (AUC) technique, which is not feasible in the clinical microbiology lab on a routine basis [104]. It was previously thought that hVISA is a precursor to VISA as selection pressure during treatment with vancomycin generates outgrowth of the VISA subpopulation [107,108], although more recent data from in vitro evolutionary

experiments suggests that may not be correct [109]. Whether hVISA in MRSAB results in increased vancomycin failure and persistent MRSAB remains debated. Some studies report worse clinical outcomes [110–116] and increased risk of persistent MRSAB [110,112–114], with others, including one systematic review and meta-analysis, showing no significant difference in mortality or persistent MRSAB [104,117–121]. Overall, the mixed data suggest that hVISA may play a role in persistent MRSAB. However, the lack of strong evidence does not necessarily justify deviating from vancomycin in routine hVISA MRSAB cases.

In summary, there is unlikely to be a single pathogen component that is individually responsible for persistence in MRSAB. The inability of the host to clear the bloodstream is likely a result of complex interplay between the bacteria, the host immune system, and the circulating antibiotic (Figure 3). Understanding characteristics of *S. aureus* increasing the probability of persistent bacteremia opens the door to novel diagnostics, which could allow for a more aggressive antibiotic strategy up-front, potentially improving patient outcomes.

Figure 3. Summary of host and pathogen factors contributing to persistent MRSAB



Treatment of persistent MRSAB

Limited high-quality evidence exists for the most effective treatment of MRSAB in general, and even less for the treatment of persistent MRSAB in particular [122].

No randomized controlled trials to date have addressed this specific question, leaving an unmet need for medical practice. However, until high-quality evidence is available, the available literature provides suggestions for best practice regarding the treatment of persistent MRSAB.

The management of MRSAB consists of three important pillars: source control, antibiotic treatment, and follow-up blood cultures. For evaluation of metastatic infection sites as targets for source control, the transesophageal echocardiogram is the most evidence based [123,124]. For positron emission tomography/computed tomography (PET-CT), there is evidence for impacting management and for reducing mortality in patients with SAB [125,126], although this latter finding may have been confounded by the introduction of immortal time bias related to including patients dying before undergoing PET-CT. Thorough clinical assessment by a trained infectious diseases consultant has been proven to be beneficial in the management of MRSAB [127]. In the case of positive follow-up blood cultures and thus persistent bacteremia despite adequate treatment, potential targets for source control must be reevaluated, and subsequently also the antibiotic therapy. This is particularly true now, as the specific antibiotic treatment options have evolved over time.

The past

For decades, vancomycin monotherapy was the only recommended antibiotic treatment for MRSAB. This was primarily due to the lack of other options for monotherapy. There has been a multiplicity of attempts to craft an effective combination antibiotic therapy for SAB. Adjunctive gentamicin appeared to be an attractive option according to in vitro data, but was associated with increased nephrotoxicity without any clinical benefit [128]. Alternatives for vancomycin, such as trimethoprim-sulfamethoxazole, did not achieve non-inferiority for the treatment of MRSAB [18,129]. For many years, the addition of rifampin was thought to improve outcomes, but the ARREST trial has ruled out that hypothesis: outcomes in both MSSA and MRSAB did not improve with adjunctive rifampin [130].

Historically, there were few options for treatment of persistent MRSAB. When confronted with persistent MRSAB > 7 days after vancomycin initiation and a MIC of 2 µg/mL, almost three-quarters of surveyed American ID consultants in 2005 would continue vancomycin and add another drug, usually rifampin or gentamicin. Less than 20% would switch to another agent [131]. Rather than clinical inertia, this approach was likely a consequence of the paucity of agents with proven efficacy for SAB. This changed in 2006, when daptomycin was proven to be non-inferior to vancomycin in the treatment of MRSAB [19].

The present

Following the non-inferiority trial in 2006, the U.S. guideline included daptomycin as first-choice therapy, comparable to vancomycin, for MRSAB in 2011 [10,19]. Although daptomycin monotherapy was shown to be non-inferior to vancomycin for treatment of MRSAB, the possibility of treatment-emergent resistance and treatment failure has become apparent over time [132,133]. Therefore, it is often recommended to add a second antibiotic agent to daptomycin (e.g., trimethoprim-sulfamethoxazole) with the goal of preventing daptomycin resistance from emerging, especially if source control is not achieved [10]. In Europe and the UK, the only first-choice agent in the guidelines remains vancomycin [134,135]. However, when the MIC is 2 µg/mL or higher, vancomycin is believed to be less effective, and alternative treatment options should be considered.

Multiple mono- or combination therapy options for the treatment of MRSAB have been studied in the last decade. One promising concept was the combination of vancomycin or daptomycin with an anti-staphylococcal beta-lactams (ASBLs) such as nafcillin or flucloxacillin. This clinical approach was based on exciting in vitro data demonstrating the synergy with both vancomycin and daptomycin when an ASBL was added. The CAMERA2 trial addressed this question by randomizing MRSAB patients to receive either standard therapy (daptomycin or vancomycin) or standard therapy with the addition of an ASBL. While the proportion of patients with persistent *S. aureus* bacteremia at day five was significantly lower in the combination therapy group, all-cause mortality was not significantly different and combination therapy was associated with a significantly increased rate of acute kidney injury [136]. However, whether this is true for all beta-lactams and for all patient categories has not yet been clarified [137]. The DASH trial, which enrolled only MSSA bacteremia patients, demonstrated that the addition of daptomycin to anti-staphylococcal beta-lactam did not reduce the duration of bacteremia, 90-day mortality, or rate of recurrence [138].

Ceftaroline is a fifth-generation cephalosporin with robust activity against MRSA due to its unique ability to bind with high affinity to PBP-2a [139]. It is FDA approved for the treatment of community-acquired pneumonia and acute bacterial skin and skin structure infections (including those with concurrent bacteremia) but is frequently used off-label, either alone or in combination with another antibiotic, as a treatment for MRSAB. The combination of daptomycin and ceftaroline, especially when initiated early in the disease course, is possibly associated with reduced in-hospital mortality compared to monotherapy with vancomycin or daptomycin [140–142]. Although we are lacking high-quality data to support such an approach, ceftaroline is commonly used in clinical practice in combination with vancomycin or daptomycin to treat persistent MRSAB [143,144]. There are several observational studies showing expedited bacterial clearance when deployed as a salvage therapy in refractory

MRSAB, but the effect on mortality remains unclear [145–149]. Fortunately, a large, well-designed Phase 3 randomized clinical trial that tested ceftobiprole, another cephalosporin with efficacy against MRSA, has recently completed enrollment and reported positive topline results (discussed later).

The emergence of possible alternatives for the treatment of MRSAB has an effect on the decisions that physicians make in clinical practice. In contrast to the situation in 2005, a second survey in 2017 showed that less than 20% of the surveyed American ID consultants would continue vancomycin and simply add another agent in case of persistent MRSAB on day 6. Instead, more than half of them would switch to another agent (either a single agent or daptomycin with a second agent) [150].

Although there is much (clinically unsubstantiated) debate about the most appropriate therapeutic modification in patients with persistent MRSAB, the single most important management component of these patients remains adequate source control. In the suggested management algorithm for MRSAB by Holland et al., a single positive follow-up blood culture represents a "worry point", prompting reevaluation of potential sites of metastatic infection [21]. If blood cultures continue to be positive at the 3–5-day point despite appropriate antibiotic therapy, Holland et al. presume the patient has experienced monotherapy failure and recommend the addition of ceftaroline to vancomycin or a change of therapy to daptomycin plus a second antibiotic. The recommendation to add a second antibiotic to daptomycin or vancomycin, while unproven, is primarily to thwart the development of treatment-emergent daptomycin resistance rather than to improve efficacy based upon data using simulated vegetations [151].

The future

There are a handful of clinical trials investigating future therapeutics for the treatment of MRSAB. Ceftobiprole is another fifth-generation cephalosporin currently under investigation with activity against MRSA [152,153]. Its safety and efficacy were recently evaluated in a landmark clinical trial. The ERADICATE trial is the largest clinical trial to evaluate a new antibiotic for complicated SAB and the first double-blind, placebo-controlled Phase 3 ever conducted for that indication [154]. Results were presented at IDWeek2022. Topline data from the ERADICATE trial indicate that ceftobiprole met its primary efficacy endpoint without significant obvious toxicity concerns.

Dalbavancin is approved for use in *S. aureus* bacterial skin infections, with the great advantage of having a uniquely long half-life [155]. A potential role of dalbavancin in endovascular infections has not yet been established [156]. The superiority of dalbavancin compared to standard parenteral antibiotic therapy for the completion of treatment is currently being studied in patients with complicated SAB in a phase

2b randomized clinical trial (DOTS trial) [157]. A potential role for dalbavancin in persistent bacteremia naturally warrants more follow-up research.

Driven by the lack of major breakthroughs in antibiotic treatment to improve clinical outcomes in SAB, new nonantibiotic antimicrobial modalities are an increasing subject of research. Exebacase, an anti-staphylococcal lysin, as an addition to standard-of-care antibiotics, led to a higher clinical response rate in patients with MRSAB in a proof-of-concept study [158]. A subsequent randomized trial addressing the superiority of exebacase in addition to standard-of-care antibiotics in both MSSA and MRSAB (DISRUPT trial) was terminated early for futility, following interim efficacy analysis [159]. A second anti-staphylococcal lysin, LSVT-1701, showed reduced bacterial bioburden in MRSA animal studies and demonstrated a good safety profile in a Phase I study in healthy human subjects [160]. In June 2022, further development of this asset was terminated by Roivant Sciences. Furthermore, bacteriophage therapy as an adjunctive intravenous therapy for SAB patients is currently being investigated. It was shown to be well tolerated in a group of 13 patients with severe *S. aureus* infections, including endocarditis and septic shock [161]. The diSArm trial is a phase 1b/2a randomized trial on the efficacy and safety of adjunctive bacteriophage therapy in SAB patients, which is estimated to be completed at the end of 2023 [162].

In conclusion, the unfavorable safety profiles of many combinations of antibiotics have prevented them from replacing vancomycin as the most frequently used antibiotic treatment in MRSAB. High-dose daptomycin (with a second antibiotic agent to prevent treatment-emergent resistance) and the addition of ceftaroline are currently the best practice in persistent MRSAB. Future treatment options may include dalbavancin, ceftobiprole, and novel non-antibiotic agents such as bacteriophages.

Conclusions

Persistent MRSAB is a devastating and complex disease. Understanding the interaction between host and pathogen is crucial to the challenge of improving patient outcomes. Given the lack of major breakthroughs in patient outcomes in the last decades, there seems to be a need for novel diagnostics and treatment options. Trials on genetics, biomarkers, and novel non-antibiotic agents in persistent MRSAB should be encouraged, as well as the implementation in daily practice of those that were successful. Meanwhile, it is promising that antibiotic agents such as dalbavancin [157] and ceftobiprole [154] are being studied in randomized clinical trials for SAB. These new high-quality studies represent an important step towards better understanding and ultimately improving clinical outcomes in patients with SAB.

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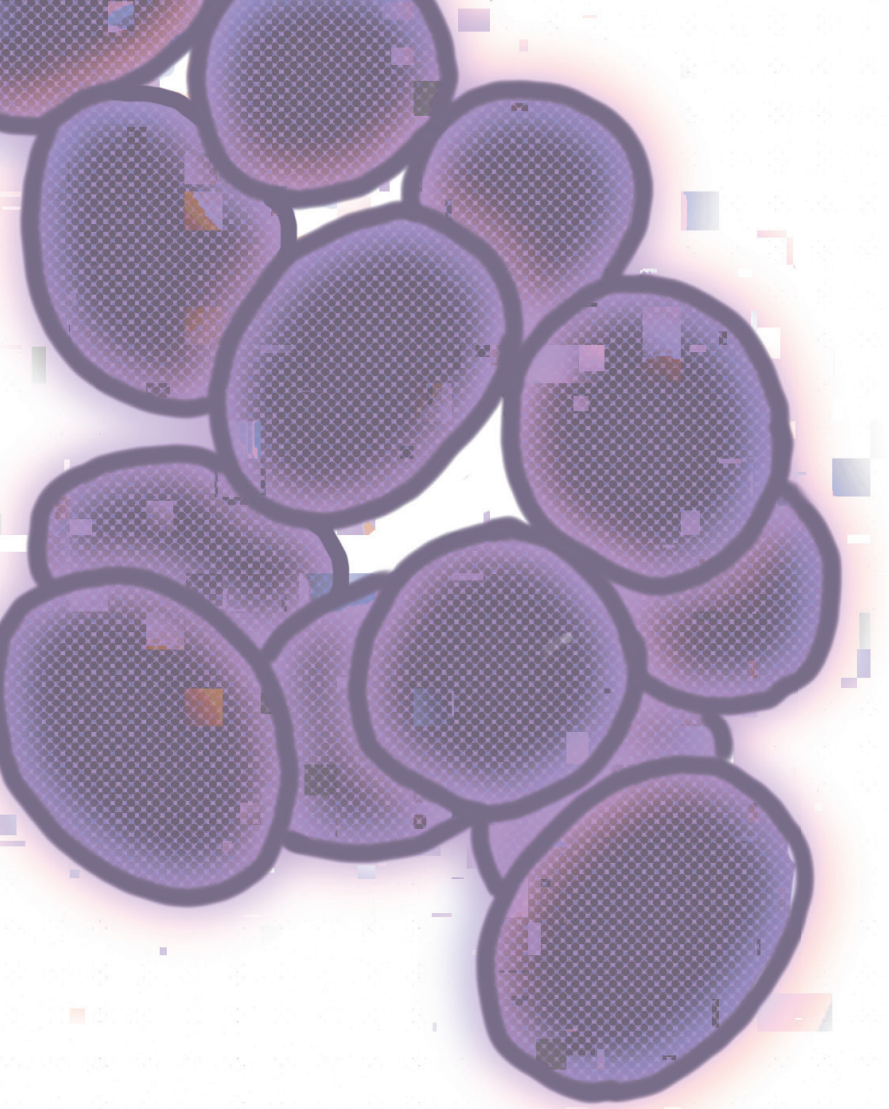

Chapter 9

The association of female sex with management and mortality in patients with *Staphylococcus aureus* bacteremia

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Abstract

Objectives

The association of biological female sex with outcome in patients with *Staphylococcus aureus* bacteraemia remains unresolved. The aim of this study was to determine the independent association of female sex with management and mortality in patients with *S. aureus* bacteraemia.

Methods

This is a post hoc analysis of prospectively collected data from the *S. aureus* Bacteraemia Group Prospective Cohort Study. Adult patients with monomicrobial *S. aureus* bacteraemia at Duke University Medical Center were enrolled from 1994 to 2020. Univariable and multivariable Cox regression analyses were performed to assess differences in management and mortality between females and males.

Results

Among 3384 patients with *S. aureus* bacteraemia, 1431 (42%) were women. Women were, as compared with men, more often Black (581/1431 [41%] vs. 620/1953 [32%], $p < 0.001$), haemodialysis dependent (309/1424 [22%] vs. 334/1940 [17%], p 0.001) and more likely to be infected with methicillin- resistant *S. aureus* (MRSA) (697/1410 [49%] MRSA in women vs. 840/1925 [44%] MRSA in men, p 0.001). Women received shorter durations of antimicrobial treatment (median 24 [interquartile range 14-42] vs. 28 [interquartile range 14-45] days, p 0.005), and were less likely to undergo transesophageal echocardiography as compared with men (495/1430 [35%] vs. 802/1952 [41%], $p < 0.001$). Despite these differences, female sex was not associated with 90-day mortality in either univariable (388/1431 [27%] in women vs. 491/1953 [25%] in men, p 0.204) or multivariable analysis (adjusted hazard ratio for women 0.98 [95% CI, 0.85-1.13]).

Discussion

Despite significant differences in patient characteristics, disease characteristics, and management, women and men with *S. aureus* bacteraemia have a similar mortality risk.

Introduction

Staphylococcus aureus, a major cause of bloodstream infections, is associated with high morbidity and mortality [1,2]. Previous studies have reported conflicting results regarding sex-related differences in *S. aureus* bacteraemia (SAB). Some [3-7], but not all [8-10], previous studies have reported higher mortality rates in women with SAB compared with men. Sex-related differences in outcome may be because of a variety of social or biological factors. For example, in a superantigen-mediated model of toxic shock using human leukocyte antigen (HLA) class II transgenic mice, women were more susceptible to lethal toxic shock caused by *S. aureus* enterotoxin B [11]. Alternately, previous cohort studies may simply have been limited by small sample size and study design. As a result, the true interaction between sex and outcome among patients with SAB is unknown.

The primary aim of this study was to determine the independent association of female sex with mortality in patients with SAB. Next, we sought to identify differences in patient, disease and management characteristics between women and men. The large study size and detailed prospective data collection, including bacterial genotyping provided the unique possibility to address the ongoing controversy on sex differences in SAB.

Methods

Study population

This is a post hoc analysis of prospectively collected data from the *S. aureus* Bacteraemia Group Prospective Cohort Study (SABG- PCS). Adult (2:18 years), hospitalized, non-neutropenic (neutrophil count >1 x 109/L) patients with monomicrobial SAB at Duke University Medical Center were enrolled from 1994 to 2020. Beginning in 2001, written informed consent was obtained from patients or their legal representatives to comply with Health Insurance Portability and Accountability Act regulations. If a patient died before the notification of their blood culture results, the patient was included using an institutional review boardeapproved Notification of Decedent Research. From March until September 2020, because of the COVID-19 pandemic, enrolment was temporarily paused. If a patient experienced multiple SAB episodes, only the initial episode was included. Follow-up was done through participants' medical records assessment at 90 days after first positive blood culture for all patients. Both clinical and microbiological data are collected in the SABG-PCS. Enrolment and data collection methods have been published previously [2].

Definitions

Sex was defined as biological sex assigned at birth [12]. The following sources were considered primary endovascular infection: central venous catheters, arterio-venous fistulas, subcutaneous catheters, intracardiac devices and endovascular grafts [13]. The route of acquisition was classified as hospital-acquired, healthcare-associated or community-acquired as previously defined [14]. The duration of symptoms was defined as the time from the patient-reported onset of symptoms to the day of first positive blood culture. Recurrent SAB after this first episode was defined as a second episode of SAB after resolution of this first and occurring at least 14 days after the last positive blood culture associated with this episode [15]. Persistent bacteraemia was defined as ≥ 3 days of positive blood cultures after appropriate treatment was initiated [2]. Patients were considered to have a hematogenous metastatic infection if they exhibited any of the following conditions during their hospitalization for SAB: infective endocarditis, vertebral osteomyelitis, septic arthritis, septic emboli, septic thrombophlebitis or deep tissue abscess [2]. Main antibiotic regimen was defined as the primary antibiotic used for definitive treatment of the episode of SAB.

Bacterial genotyping

The *S. aureus* isolates from the first blood culture obtained from enrolled patients underwent spa genotyping and further analyses to determine USA300 clone as previously described [2,16].

Outcome measures and statistical analysis

The primary study outcome was 90-day mortality, stratified by sex. The time count started from the day of the first positive blood culture. Secondary outcomes were 30-day mortality, and differences in patient, disease and management characteristics between women and men. Data were presented as counts plus percentages or proportions for categorical variables and as medians plus interquartile ranges (IQR) for continuous variables. Fisher's exact, Chi-square and Mann-Whitney U tests were used to analyse differences in patient and disease characteristics. Survival curves were constructed using the Kaplan-Meier method. Cox regression analysis was used to assess the independent effect of female sex on mortality. Variables with $p < 0.01$ in univariable analysis and clinically relevant variables were added to the multivariable analysis. To evaluate differences in subgroups, mortality by sex was additionally analysed for methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) separately, stratified for route of acquisition and for different time periods. All statistical analyses were performed using IBM SPSS statistics version 28.0.1.1.

Ethical approval

Ethical approval was granted by the Duke University Medical Center institutional review board.

Results

A total of 3384 patients were enrolled from 1994 to 2020. Among them, 1431 (42%) were women. Median age was 60 years in both sexes (Fig. S2). Female patients with SAB were, as compared with male patients, more frequently Black (581/1431 [41%] vs. 620/ 1953 [32%], p < 0.001), more often haemodialysis dependent (309/ 1424 [22%] vs. 334/1940 [17%], p 0.001), more likely to have implanted foreign material (817/1422 [58%] vs. 1014/1949 [52%], p 0.002) and more likely to have used corticosteroids in the past month (315/1422 [22%] vs. 355/1933 [18%], p 0.008, Table 1). By contrast, men more frequently had a history of injection drug use (142/1933 [7%] vs. 64/1422 [5%], p 0.001) and experienced higher rates of metastatic infection (813/1952 [42%] vs. 512/1431 [36%], p 0.001).

Microbiological characteristics

Women were more likely to be infected with MRSA as opposed to MSSA, compared with males (697/1410 [49%] MRSA in female patients vs. 840/1925 [44%] MRSA in male patients, p 0.001). In the 3136 isolates that were genotyped, 516 distinct spa types were identified, which were equally distributed between the sexes (p 0.265, Table S1). Ninety-one per cent (2599/2843) of the isolates with an identified Clonal Complex (CC) belonged to one of the six most common CCs: CC002, CC004, CC008, CC012, CC084, and CC0189, which were also similarly distributed between sexes (p 0.080, Table S2). The percentage of patients infected with the USA300 clone was equal in women and men (respectively 130/1326 and 173/1810, both 10%, p 0.854, Table 1).

Table 1. Patient and clinical characteristics stratified by sex

	All patients ***N*= 3384**	**Female patients** ***N*= 1431**	**Male patients** ***N*= 1953**	**p-value** [a]
Demographics				
Female sex, *n (%)*	1431 (42.3)	1431 (100)	0 (0)	
Age in years, *median (IQR)*	60 (47-70)	60 (47-71)	60 (48-70)	0.164
Race, *n (%)*				<0.001
White	2063 (61.0)	806 (56.3)	1257 (64.4)	
Black	1201 (35.5)	581 (40.6)	620 (31.7)	
Other	120 (3.5)	44 (3.1)	76 (3.9)	
Comorbidities, *n (%)*				
Diabetes mellitus	1296 (38.5)	562 (39.5)	734 (37.8)	0.316
Hemodialysis dependent	643 (19.1)	309 (21.7)	334 (17.2)	0.001
Organ transplant	218 (6.5)	78 (5.5)	140 (7.2)	0.047
Injection drug use	206 (6.1)	64 (4.5)	142 (7.3)	0.001
Corticosteroid use past 30 days	670 (20.0)	315 (22.2)	355 (18.4)	0.008
Foreign body present	1831 (54.3)	817 (57.5)	1014 (52.0)	0.002
Initial source of bacteremia, *n (%)*				0.037
Endovascular	912 (27.0)	421 (29.4)	491 (25.1)	
Pulmonary	319 (9.4)	136 (9.5)	183 (9.4)	
Skin/soft tissue	707 (20.9)	301 (21.0)	406 (20.8)	
Other	770 (22.8)	311 (21.7)	459 (23.5)	
Unknown	676 (20.0)	262 (18.3)	414 (21.2)	
Micro-organism, *n (%)*				
Methicillin-resistance (MRSA)	1537 (46.1)	697 (49.4)	840 (43.6)	0.001
USA300[b]	303 (9.7)	130 (9.8)	173 (9.6)	0.854
Presentation, *median (IQR)*				
Days of symptoms until diagnosis [c]	2 (1-5)	2 (1-4)	2 (1-5)	0.014
APS score	8 (5-13)	9 (5-13)	8 (5-13)	0.037

	All patients *N*= 3384	Female patients *N*= 1431	Male patients *N*= 1953	p-value [a]
Route of acquisition, *n (%)*				0.009
Hospital acquired	920 (27.2)	405 (28.4)	515 (26.4)	
Healthcare associated	1878 (55.6)	810 (56.8)	1068 (54.8)	
Community acquired	579 (17.1)	212 (14.9)	367 (18.8)	
Persistence				
Persistent bacteremia [d], *n (%)*	1269 (37.5)	517 (36.1)	752 (38.5)	0.161
No. of days positive blood cultures, *median (IQR)*	1 (1-4)	1 (1-4)	1 (1-4)	0.210
Disease management				
TTE performed, *n (%)*	2540 (75.3)	1057 (74.0)	1483 (76.2)	0.158
TEE performed, *n (%)*	1297 (38.4)	495 (34.6)	802 (41.1)	<0.001
Duration of antibiotics, *median (IQR)*	28 (14-44)	24 (14-42)	28 (14-45)	0.005
Intervention performed [e], *n (%)*	1656 (49.1)	702 (49.3)	954 (48.9)	0.834
Clinical outcomes, *n (%)*				
Metastatic infection	1325 (39.2)	512 (35.8)	813 (41.6)	0.001
Recurrent bacteremia [f]	317 (9.4)	147 (10.3)	170 (8.7)	0.135
Mortality 30 days	682 (20.2)	301 (21.0)	381 (19.5)	0.278
Mortality 90 days	879 (26.0)	388 (27.1)	491 (25.1)	0.204

Values are counts (%) for categorical variables and medians (interquartile ranges) for continuous variables. APS assessed at time of patient's first blood culture. APS, acute physiology score; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography.

a Fisher's exact, Mann-Whitney U, and Pearson Chi-Square tests were used in the analyses.

b USA300 status was missing in 248 (7%) patients. For all other variables, missing data was <3%.

c More than 14 days of symptoms was set as 14 days.

d Defined as 3 days or more of positive blood cultures.

e Whether an intervention was performed to treat the bacteraemia (e.g. surgery, drainage, line or device removal).

f Recurrent SAB means recurrence after this first SAB episode.

Medical management

Women were less likely to undergo transesophageal echocardiography (TEE) as compared with men (495/1430 [35%] vs. 802/ 1952 [41%], p < 0.001). There was no difference in transthoracic echocardiography use between sexes. Women received shorter durations of antimicrobial treatment (median 24 [IQR 14-42] vs. 28 [IQR 14-45] days, p 0.005) compared with men (Table 1 and Fig. S3). The main antibiotic regimen was similar in women and men with MRSA bacteraemia but differed significantly in MSSA bacteraemia. Male patients with MSSA bacteraemia were more often treated with cefazolin or an anti-staphylococcal penicillin, whereas female patients with MSSA bacteraemia were more often treated with other non-first-choice antibiotic regimens (p < 0.006, Table 2).

Table 2. Main antibiotic regimen for patients with MRSA and MSSA bacteraemia stratified by sex

Main antibiotic regimen	All patients	Female patients	Male patients	p-value [a]
MRSA bacteremia, *n (%)*	n = 1498	n = 679	n = 819	0.29
Vancomycin	1332 (88.9)	595 (87.6)	737 (90.0)	
Daptomycin	69 (4.6)	33 (4.9)	36 (4.4)	
Other[b]	97 (6.5)	51 (7.5)	46 (5.6)	
MSSA bacteremia, *n (%)*	n = 1746	n = 689	n = 1057	0.006
Cefazolin	842 (48.2)	318 (46.2)	524 (49.6)	
Anti-staphylococcal penicillin	380 (21.8)	135 (19.6)	245 (23.2)	
Other[b]	524 (30.0)	236 (34.3)	288 (27.2)	

Values are counts (%). Data were missing in <3%.

MRSA, methicillin-resistant *S aureus*; MSSA, methicillin-susceptible *S aureus.*

a Pearson Chi-Square tests were used for the analyses.

b Other antibiotics used in MSSA bacteraemia were mainly vancomycin, ceftriaxone and daptomycin. Other antibiotics used in MRSA bacteraemia were mainly linezolid.

Outcome

Despite differences in clinical presentation and management of SAB in women and men, no significant differences were noted in 90-day mortality in either univariable (388/1431 [27%] in women vs. 491/1953 [25%] in men, p 0.204, Table 1) or multivariable analysis (adjusted hazard ratio for women 0.98, 95% CI, 0.85-1.13, Figure 1). Thirty-day mortality was also similar in women and men (301/1431 [21%] in women vs. 381/1953 [20%] in men, p 0.278). In the patients who died within 90 days, the median time from first positive blood culture to death was similar in both sexes (median 13 [IQR 5-27] days in women vs. 12 [IQR 4-28] days in men, p 0.346, Figure 2). When stratified for MSSA versus MRSA, no difference in mortality between sexes was found in either group (Table S3). Furthermore, no significant differences in mortality between women and men were noted across study time periods (1994-2002; 2003-2011; 2012-2020, Table S4) or when analyses were stratified by route of acquisition (community-acquired, healthcare-associated or hospital-acquired SAB; Table S5).

Fig. 1. Forest plot with adjusted hazard ratios for 90-day mortality in patients with *S. aureus* bacteraemia. aHR, adjusted hazard ratio; APS, acute physiology score at time of first positive blood culture; MRSA, methicillin-resistant *Staphylococcus aureus*; TEE, transesophageal echocardiography. [a]Reference: MSSA bacteraemia treated with cefazolin or antistaphylococcalpenicillin. [b]Reference: white race. [c]Reference: community-acquired.

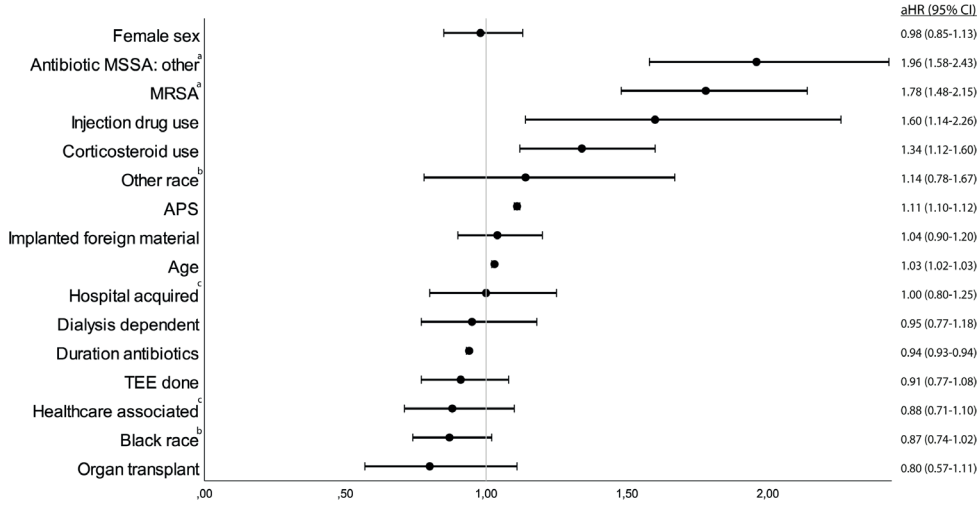
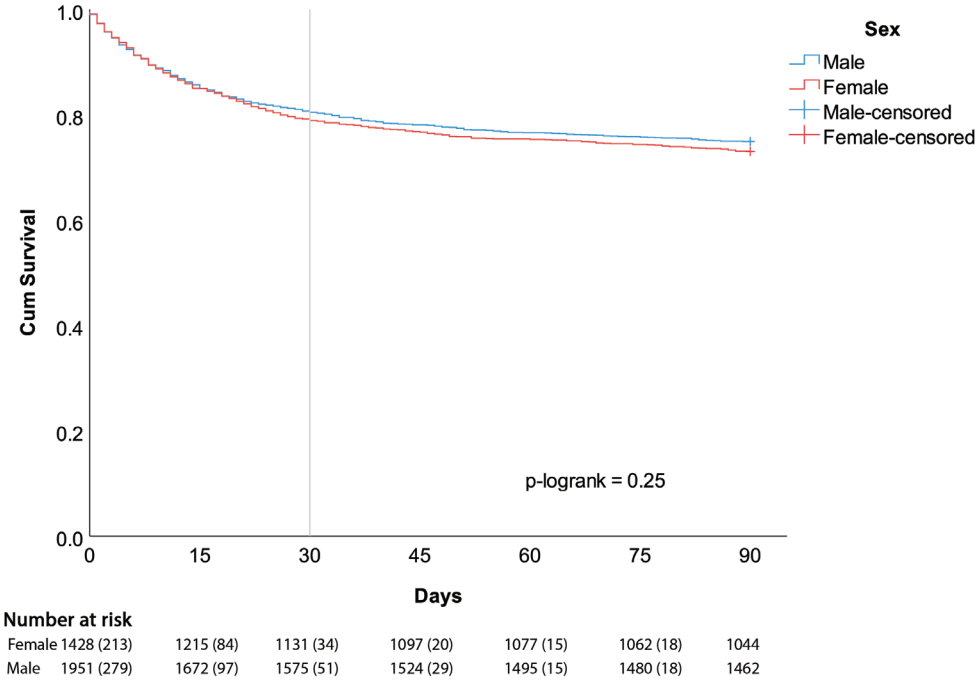


Fig. 2. Survival in female and male patients with *Staphylococcus aureus* bacteraemia. Kaplan-Meier survival curve with proportional cumulative survival of females and males with *S. aureus* bacteraemia.



Discussion

The interaction between female sex and mortality in SAB and bloodstream infections in general has been controversial for decades [6,17]. The historical tendency to include fewer female patients in scientific studies may have contributed to the knowledge gap concerning sex-specific outcomes in SAB [18].

Some, but not all [19,20], studies have reported higher rates of mortality in females with hospital-acquired bloodstream infection [21], severe sepsis [22,23] and endocarditis [24]. The previous literature on sex differences in patients with SAB is similarly contradictory (Table 3). For example, although studies from Israel [7] and Denmark [3] reported higher mortality in female patients with SAB, similar publications from Finland [9] and Korea [8] found no overall mortality difference in patients with SAB. Our study adds to this ongoing discussion by reporting on a large, prospective cohort of U.S. patients with a high prevalence of recognized risk factors for poor outcome in SAB [15,25-27].

Table 3. Summary of studies focused on sex differences in mortality in patients with *S. aureus* bacteraemia

Study	Years of patient inclusion	Country	Number of patients	MSSA or MRSA	Outcome
Forsblom et al [9] Infection 2018	1999-2002 2006-2007	Finland	617	MSSA	• No difference in 90-day mortality between sexes
Kang et al [8] CMI 2018	2009-2017	South Korea	1974	MSSA and MRSA	• No difference in overall mortality between sexes • Higher mortality in males with CCWI ≤ 3 and MRSA
Smit et al [3] CMI 2017	2000-2011	Denmark	2638	MSSA	• Higher 30-day mortality in females (29 vs 22%; aHR 1.30)
Mansur et al [7] Gend Med 2012	1988-2007	Israel	1293	MSSA and MRSA	• Higher 30-day mortality in females (45 vs 35%; OR 1.54)

Although men and women with SAB in our study had similar outcomes, their characteristics differed significantly. For example, less than half (43%) of admitted patients with SAB were female, whereas 51% of the North Carolinian population is female [28]. This suggests a lower a priori risk of SAB in female than male patients and is consistent with previous reports [27]. Although different health-seeking behaviour between sexes has been suggested [5], in our study both men and women had a median of 2 days from start of symptoms until diagnosis. Female patients had higher rates of MRSA compared with males, possibly due in part to a higher prevalence of haemodialysis dependence, healthcare exposure, corticosteroid therapy and other well-described risk factors for MRSA [29-31]. Interestingly, rates of bacteraemia with

the hypervirulent USA300 MRSA clone were similar among the two sexes despite the higher rates of MRSA infection in women overall.

Although transthoracic echocardiography use was similar between sexes, TEE was performed significantly less often in female than male patients, a finding that is consistent with previous reports [32,33]. Furthermore, a shorter median duration of antibiotics was prescribed in female compared with male patients. It is unclear whether these differences reflect a sex-driven bias in management or simply the fact that men in our study had higher rates of metastatic infection, and thus more often a true indication for TEE and prolonged therapy. Alternately, it is also possible that the higher rate of metastatic infection identified in male patients may reflect the higher rate of diagnostic testing with TEE and other modalities.

A limitation of our study is the setting: a single academic centre in a region with high MRSA prevalence, making the results less generalizable to some other settings. Our study could have been underpowered to detect a small sex difference. A large meta-analysis would be helpful to determine smaller differences. Also, only the first episode of bacteraemia was considered; therefore, a bias towards less severe SAB is possible. Another potential limitation is the long period of time during which the study was conducted, starting back in the nineties. Awareness of sex differences has increased over the years in many medical fields. However, because we found consistent results on sex differences in all time periods, this does not seem to be of important influence in our study. The increasing overall mortality over time is remarkable, and we hypothesize that the increasing tertiary care function of Duke University Hospital and the introduction of informed consent, which provides the possibility for patients to refuse participation, may be contributing factors. Finally, although sex assigned at birth was reported in the SABG-PCS, gender was not. People assigned female at birth and people identifying as women may comprise clinically distinct populations with different effects on health [12,34].

In conclusion, significant differences between females and males exist in patient, disease and management characteristics of SAB. Whereas some differences may be because of fixed biological distinctions or can be explained by different disease manifestations, others warrant further research to determine whether a sex-driven bias exists. Despite the multiple differences, women and men in this large cohort of patients with SAB have a similar mortality risk.

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Supplementary data

Figure S1. Flow diagram study participants

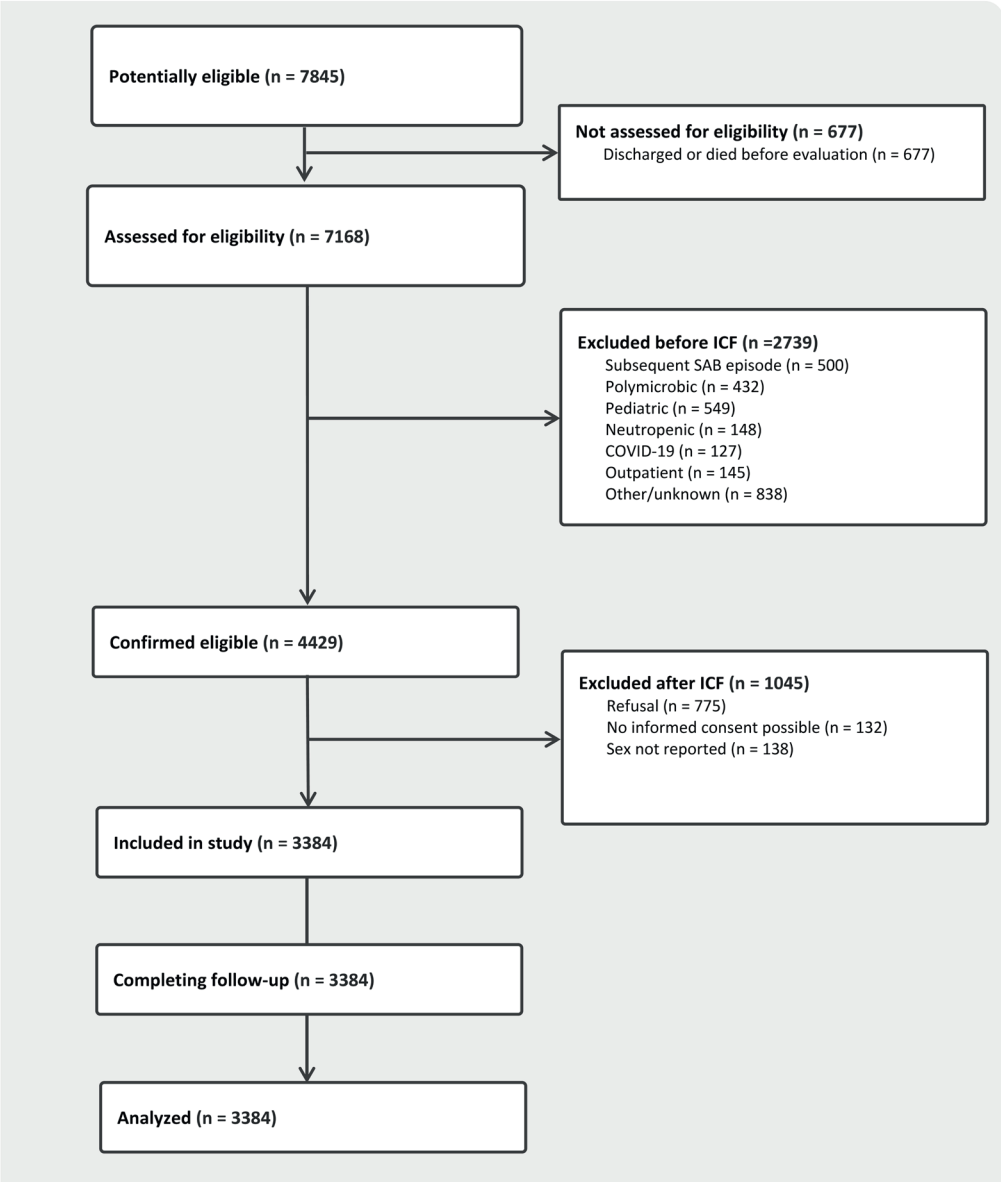
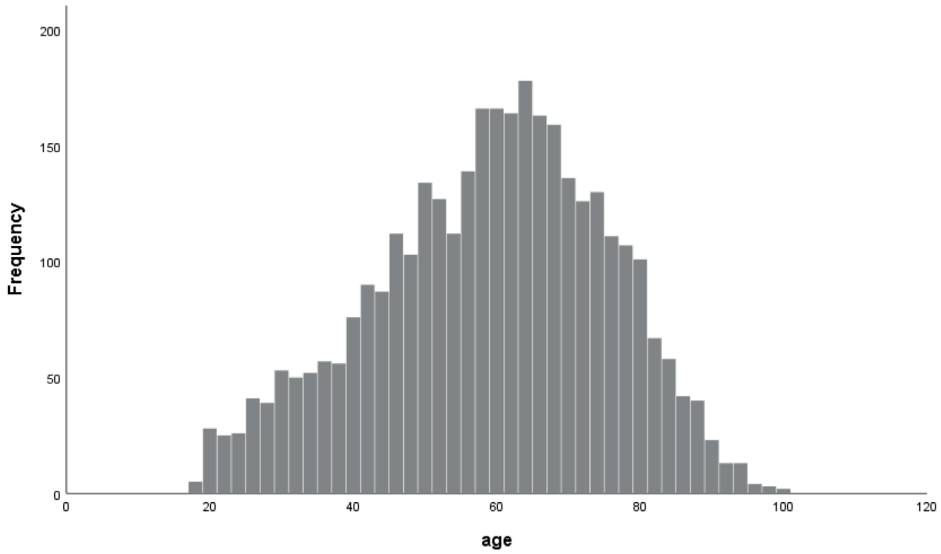
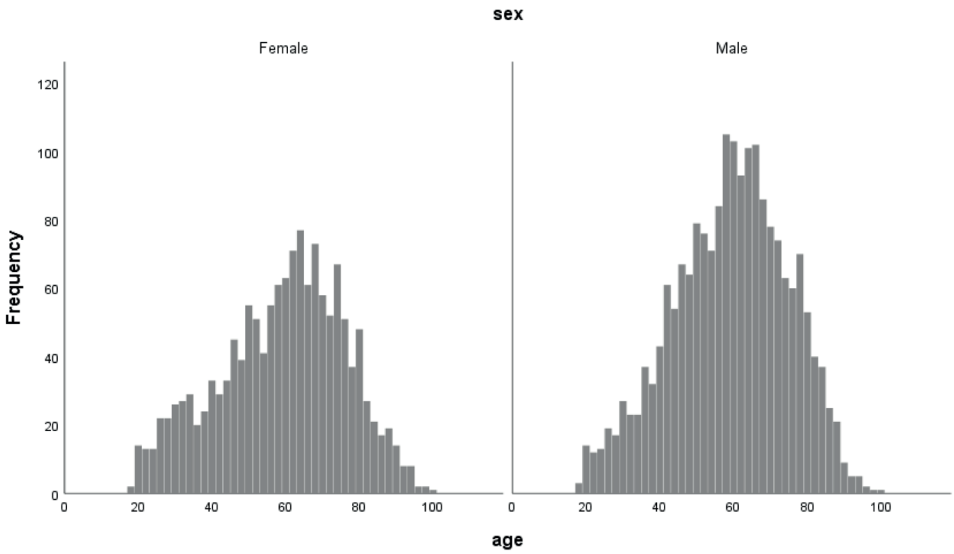


Figure S2. Distribution of age in females and males

A.



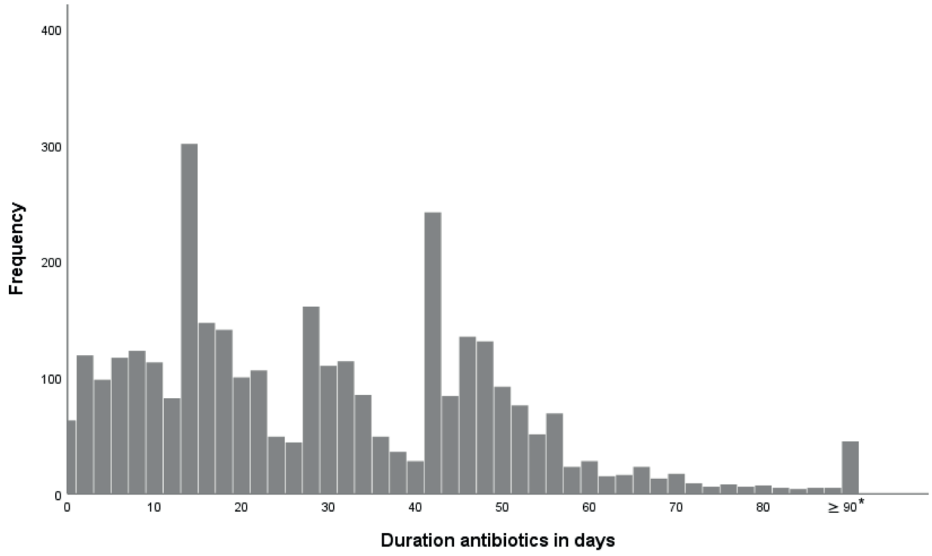
B.



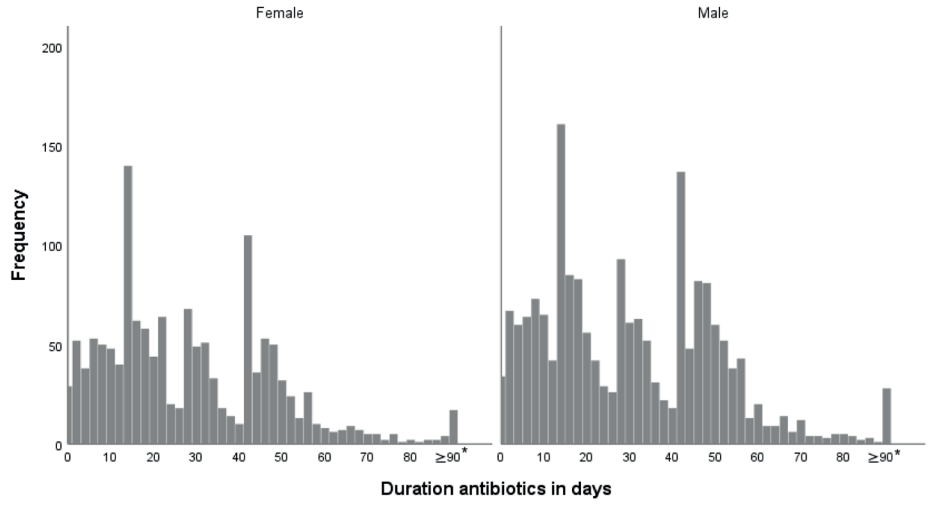
Legend. Distribution of age in years in all patients (A) and stratified by sex (B).

Figure S3. Distribution of antibiotic duration in females and males

A.



B.



Legend. Distribution of antibiotic duration in days in all patients (A) and stratified by sex (B). *Patients receiving long-term suppressive antibiotics and others found to still be on antibiotics at day 90 were included in ≥ 90 days.

Table S1. Available online.

Table S2. Genotype data – Clonal Complexes (CC)

		Male	Female	Total
CC	2	476	409	885
	4	91	83	174
	5	3	2	5
	8	449	302	751
	9	0	1	1
	12	283	214	497
	15	18	6	24
	30	2	1	3
	72	0	1	1
	84	69	50	119
	148	29	14	43
	150	1	2	3
	164	9	10	19
	189	112	61	173
	193	6	5	11
	213	7	11	18
	216	43	24	67
	267	20	8	28
	324	11	9	20
	398	1	0	1
Missing/unknown		180	113	293
Total		1810	1326	3136

Table S3. Mortality in females and males with *S. aureus* bacteremia, stratified by MSSA vs MRSA.

	MSSA			MRSA		
	Females N=713	Males N=1085	p	Females N=697	Males N=840	p
Mortality 30-day	133 (18.7)	186 (17.1)	0.41	166 (23.8)	192 (22.9)	0.67
Mortality 90-day	160 (22.4)	238 (21.9)	0.82	225 (32.3)	250 (29.8)	0.29

Legend. Values are counts (%). Data was missing in <3%.

Table S4. Mortality, TEE performance and antibiotic regimen in MSSA bacteremia in females and males with *S. aureus* bacteremia, stratified by time period.

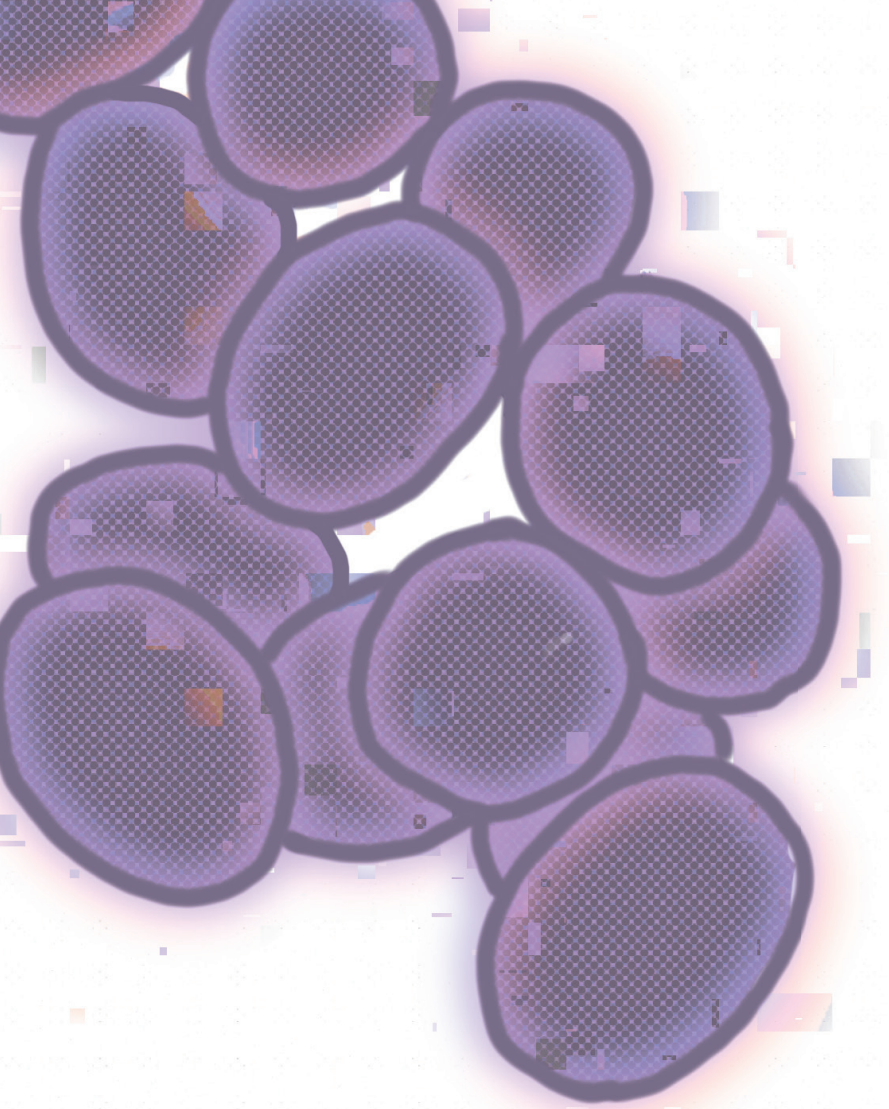
	1994-2002			2003-2011			2012-2020		
	Females N=497	Males N=630	p	Females N=422	Males N=485	p	Females N=512	Males N=838	p
Mortality 30-day	87 (17.5)	91 (14.4)	0.16	82 (19.4)	96 (19.8)	0.93	132 (25.8)	194 (23.2)	0.29
Mortality 90-day	122 (24.5)	138 (21.9)	0.32	102 (24.2)	117 (24.1)	1.00	164 (32.0)	236 (28.2)	0.14
TEE performed	179 (36.1)	251 (39.9)	0.20	116 (27.5)	168 (34.6)	0.02	200 (39.1)	383 (45.7)	0.02
Main antibiotic regiment in MSSA			0.08			0.22			0.35
Cefazolin	108 (43.7)	169 (49.0)		60 (33.9)	81 (36.7)		150 (56.6)	274 (55.8)	
Anti-staphylococcal penicillin	48 (19.4)	79 (22.9)		44 (24.9)	67 (30.3)		43 (16.2)	99 (20.2)	
Other[b]	91 (36.8)	97 (28.1)		73 (41.2)	73 (33.0)		72 (27.2)	118 (24.0)	

Legend. Values are counts (%). Data was missing in <3%. [a] Pearson Chi-Square tests were used for the analyses. [b]Other antibiotics used in MSSA bacteremia were mainly vancomycin, ceftriaxone and daptomycin.

Table S5. Mortality in females and males with *S. aureus* bacteremia, stratified by route of acquisition.

	Community-acquired			Healthcare-associated			Hospital-acquired		
	Females N=212	Males N=367	p	Females N=810	Males N=1068	P	Females N=405	Males N=515	p
Mortality 30-day	38 (17.9)	73 (19.9)	0.59	158 (19.5)	181 (16.9)	0.16	105 (25.9)	127 (24.7)	0.70
Mortality 90-day	48 (22.6)	91 (24.8)	0.61	203 (25.1)	234 (21.9)	0.11	137 (33.8)	166 (32.2)	0.62

Legend. Values are counts (%). Data was missing in <3%.



Chapter 10

Female sex and mortality in patients with *Staphylococcus aureus* bacteremia: a systematic review and meta-analysis

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Abstract

Importance. *Staphylococcus aureus* is the leading cause of death due to bacterial bloodstream infection. Female sex has been identified as a risk factor for mortality in *S aureus* bacteremia (SAB) in some studies, but not in others.

Objective. To determine whether female sex is associated with increased mortality risk in SAB.

Data sources. MEDLINE, Embase, and Web of Science were searched from inception to April 26, 2023.

Study selection. Included studies met the following criteria: (1) randomized or observational studies evaluating adults with SAB, (2) included 200 or more patients, (3) reported mortality at or before 90 days following SAB, and (4) reported mortality stratified by sex. Studies on specific subpopulations (eg, dialysis, intensive care units, cancer patients) and studies that included patients with bacteremia by various microorganisms that did not report SAB-specific data were excluded.

Data extraction and synthesis. Data extraction and quality assessment were performed by 1 reviewer and verified by a second reviewer. Risk of bias and quality were assessed with the Newcastle-Ottawa Quality Assessment Scale. Mortality data were combined as odds ratios (ORs).

Main outcome and measures. Mortality at or before 90-day following SAB, stratified by sex.

Results. From 5339 studies retrieved, 89 were included (132 582 patients; 50 258 female [37.9%], 82 324 male [62.1%]). Unadjusted mortality data were available from 81 studies (109 828 patients) and showed increased mortality in female patients compared with male patients (pooled OR, 1.12; 95% CI, 1.06-1.18). Adjusted mortality data accounting for additional patient characteristics and treatment variables were available from 32 studies (95 469 patients) and revealed a similarly increased mortality risk in female relative to male patients (pooled adjusted OR, 1.18; 95% CI, 1.11-1.27). No evidence of publication bias was encountered.

Conclusions and relevance. In this systematic review and meta-analysis, female patients with SAB had higher mortality risk than males in both unadjusted and adjusted analyses. Further research is needed to study the potential underlying mechanisms.

Introduction

Staphylococcus aureus is the leading cause of death due to bacterial bloodstream infection [1]. Previously identified risk factors for mortality in patients with *Staphylococcus aureus* bacteremia (SAB) have included increasing age, infective endocarditis, hemodialysis dependence, and persistent bacteremia, among others [2]. Female sex has been suggested as risk factor for mortality in SAB in several studies, with an increase of mortality of up to 30% relative to male patients [3-5]. However, other studies found no sex inequality in outcome of SAB [6,7], or even a higher mortality in male individuals in a subgroup of patients with a higher comorbidity score [8]. Thus, the impact of female sex in SAB remains unclear. The aim of this systematic review and meta-analysis was to determine whether female sex is associated with mortality in SAB.

Methods

The key question of this systematic review was: is female sex associated with increased mortality risk in patients with SAB? The study protocol was registered on Prospero (CRD42022373176). We followed the meta-analysis of observational studies in epidemiology Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guideline as the included studies involved observational data.

Search strategy

We conducted a literature search of MEDLINE via PubMed, Embase via Elsevier, and Web of Science Core Collection (1900 to present) via Clarivate from inception to October 31, 2022, using a combination of key words to capture *S aureus*, bacteremia, mortality, and sex (eAppendix 1 in Supplement 1). An experienced medical librarian (S.K.) devised, developed, and executed the search with input from the entire team. The search was peer reviewed by a second medical librarian according to a modified Peer Review of Electronic Search Strategies (PRESS) checklist [9]. No limitations were placed on language in the initial search, but studies published in languages other than English were excluded in the full-text review phase. A search update was conducted on April 26, 2023, to identify newly published studies. In addition, we hand-searched key references to identify citations not captured in the electronic database searches. All results were compiled in EndNote and imported into Covidence, a web-based data synthesis software program [10], for deduplication and screening.

Study selection, data extraction, and quality assessment

We included studies that met the following conditions: (1) randomized or observational study evaluating outcomes in adults with SAB, (2) included 200 or more patients, (3) reported mortality at or before 90 days following SAB, and (4) reported mortality stratified by sex. Exclusion criteria were studies on specific subpopulations (eg, dialysis, intensive care unit, hematological or oncological patients), studies that included SAB patients as a subgroup (eg, patients with bacteremia by any microorganism) that did not report SAB-specific data, and studies using (partially) the same cohort as another study included in this review. In this latter scenario, the study with the largest cohort was included. Titles and abstracts of articles (with authors and institutions visible) identified through our primary search were screened independently by two reviewers (A.W. reviewed all; R.K., M.W., J.K., F.R., J.P., S.M., S.K., M.L., V.F., and J.T. were second reviewers). Conflicts at this stage were resolved by a third person. Articles marked for full-text review underwent full-text screening by two independent reviewers. Conflicts at this stage were resolved by consensus or by obtaining a third reviewer's opinion when consensus could not be reached. Data extraction and quality assessment was done by one reviewer and verified by a second reviewer. Extracted variables included lead author, journal, year of publication, start and end year of inclusion, country, aim of study, study design, number of hospitals, number of patients, population description, and whether methicillin-resistant *S aureus* (MRSA), methicillin-susceptible *S aureus* (MSSA), or both were addressed. Unadjusted mortality stratified by sex was extracted, as well as adjusted mortality when reported, the statistical model and the covariates for which mortality was adjusted. If a study described mortality for two subgroups (eg, for MSSA and MRSA bacteremia separately), both were included. Risk of bias and quality were assessed with the Newcastle-Ottawa Quality Assessment Scale [11] (eAppendix 2 in Supplement 1) because only observational studies were identified.

Statistical analysis

Mortality data were combined as odds ratios (ORs). If ORs were not reported in a study, we calculated ORs from raw mortality by sex if such data was available. If raw data was not available either, then ORs were calculated from the provided risk ratio (RR) or hazard ratio (HR) values based on previously published methods [12,13]. In the single study that reported a rate ratio [14], this rate ratio was used to estimate the OR [15]. Sensitivity analyses involving only studies that directly reported an OR (as opposed to estimating OR based on HR or RR) were conducted. ORs were combined using inverse variance with random effects models. We used the Knapp and Hartung method to adjust the standard errors of the estimated coefficients [16,17]. Robustness of findings were assessed through influence and sensitivity analyses as detailed in the

text. We evaluated statistical heterogeneity with the Cochran Q and I2 statistics. To explore potential sources of heterogeneity, we performed meta-analyses on subsets of studies to determine if variation in factors such as mortality time point (eg, 30-day vs 90-day mortality), bacterial groups (eg, MSSA only, MRSA only, both MSSA and MRSA), or geographic location between studies could be contributing. Statistical analyses were performed with RStudio version 2022.02.0 (R Project for Statistical Computing). Publication bias was assessed using funnel plots with the Egger test [18] when ten or more studies were included in the analysis. We used the Evidence-based Practice Center (EPC) model from the US Agency for Healthcare Research and Quality (AHRQ) to grade overall strength of evidence [19]. A full description of the EPC approach is detailed in eAppendix 3 in Supplement 1.

Results

We screened the title and abstract of 5339 studies, and 4778 were deemed irrelevant (Figure 1). A full-text assessment was performed on 561 studies, and 472 of these were excluded. We included 89 studies in the analysis, with a total of 132 582 patients (50 258 female [37.9%], 82 324 male [62.1%]) (Table) [3-8,14,20-101]. All data on mortality by sex were from observational studies: 88 of 89 cohort studies and one post hoc analysis of a randomized clinical trial. Mortality was most frequently assessed at 28 to 30 days (54 of 89 studies [61%]). The majority of studies were conducted in Europe (36 [40%]), Asia (24 [28%]) and North America (20 [22%]). The majority of studies were published after 2010 (68 [76%]). Thirty-two studies (36%) were rated as having low risk of bias, and 57 studies (64%) as having high risk of bias (detailed quality assessment of each study in eTable 1 in Supplement 1).

Mortality by sex

Unadjusted mortality data was available from 81 studies (109 828 patients) and revealed an increased mortality risk in female compared with male patients (pooled OR, 1.12; 95% CI, 1.06-1.18) (Figure 2). Moderate heterogeneity was observed in this analysis ($Q = 130.17$; $P < .001$; $I2 = 37\%$). An influence analysis revealed that exclusion of any single study did not significantly alter the findings from the overall cohort (eAppendix 4 in Supplement 1). A sensitivity analysis with only studies that had an OR that was either reported or could be directly calculated (ie, excluding 14 studies in which RR or HR were reported) similarly did not change the overall findings (eFigure 1 in Supplement 1). Exclusion of single-center studies did not change the overall findings. No funnel plot asymmetry was found (eFigure 2 in Supplement 1).

Figure 1. Search flow diagram of systematic review

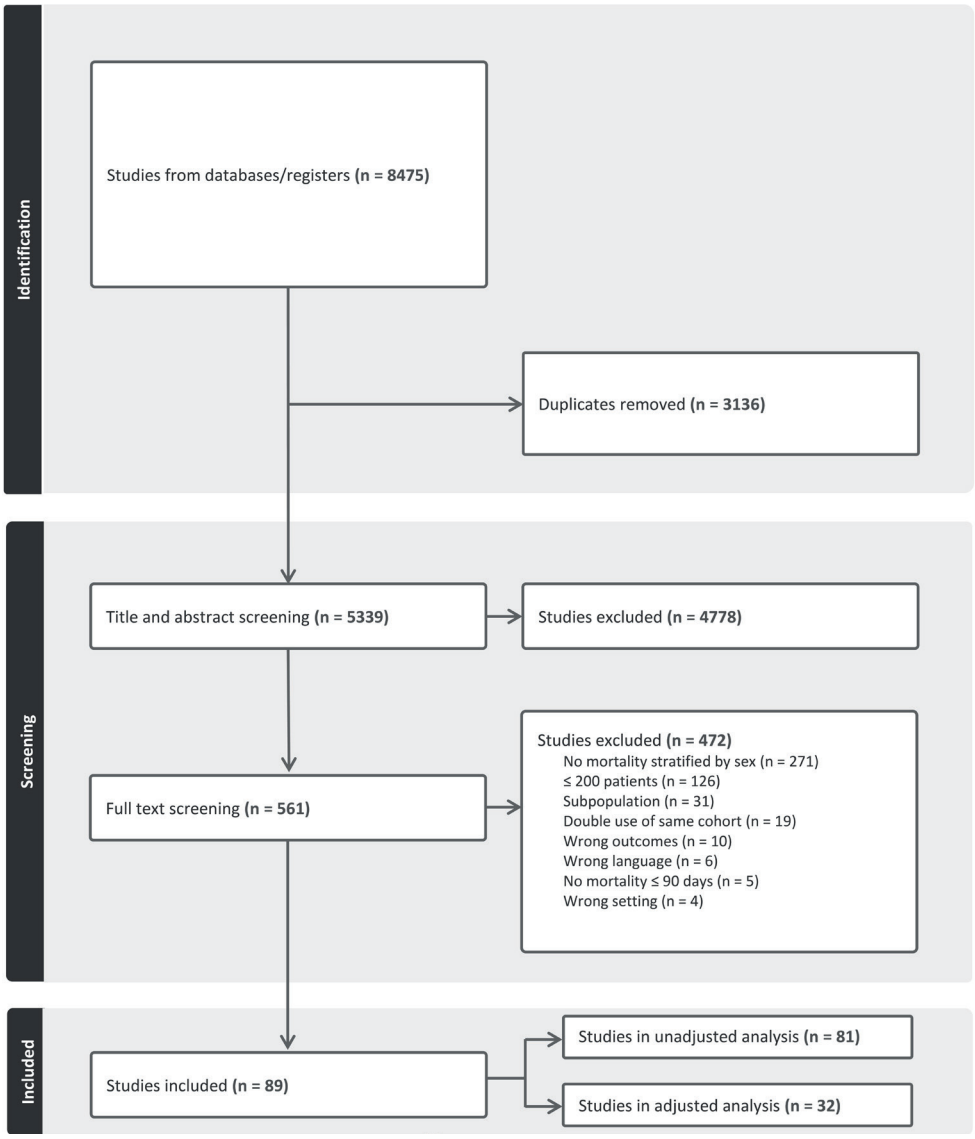


Table 1 (next page). Description of studies included in systematic review.

	Number of studies (%) N= 89
Publication year	
2000-2010	21 (24)
2011-2023	68 (76)
Study design	
Cohort study	88 (99)
Post-hoc analysis randomized trial	1 (1)
Continent	
Europe	36 (40)
Asia	24 (27)
North America	20 (22)
Oceania	5 (6)
South America	1 (1)
Africa	1 (1)
Multiple	2 (2)
Number of hospitals included	
1	44 (49)
2-20	33 (37)
>20	13 (15)
Number of patients included	
200 – 1,000	69 (78)
1,000 – 10,000	15 (17)
>10,000	4 (4)
Population	
All SAB patients	82 (92)
Healthcare/hospital-associated SAB	3 (3)
Community-acquired SAB	4 (4)
Outcome measure	
7 day mortality	1 (1)
14 day mortality	4 (4)
28-30 day mortality	54 (61)
90 day mortality	9 (10)
In-hospital mortality	16 (18)
Attributable mortality	5 (6)
MRSA vs MSSA	
Both MRSA and MSSA	59 (66)
Only MRSA	20 (22)
Only MSSA	10 (11)

Figure 2. Forest Plot of Unadjusted Mortality in Female vs Male Patients With *Staphylococcus aureus* Bacteremia

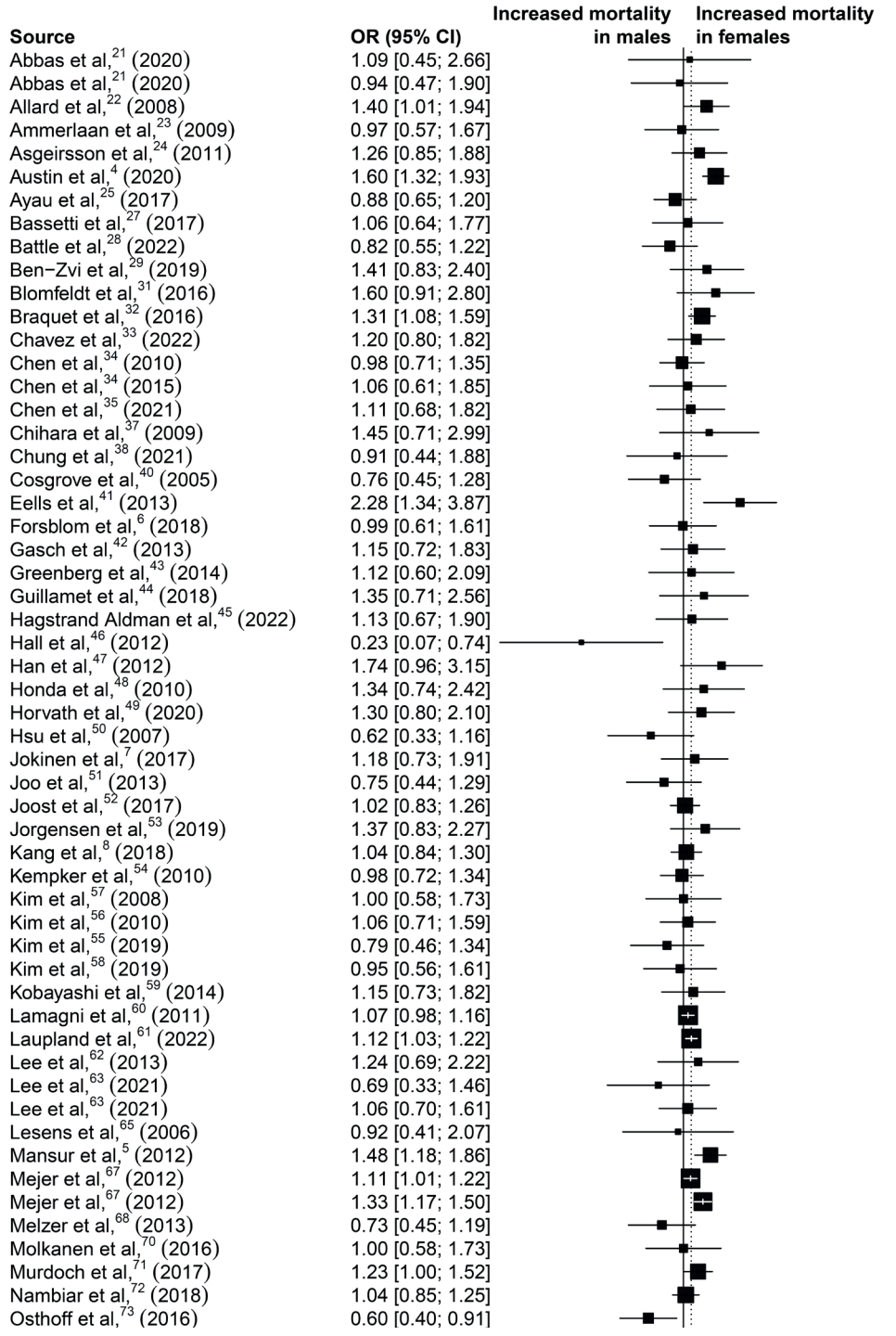
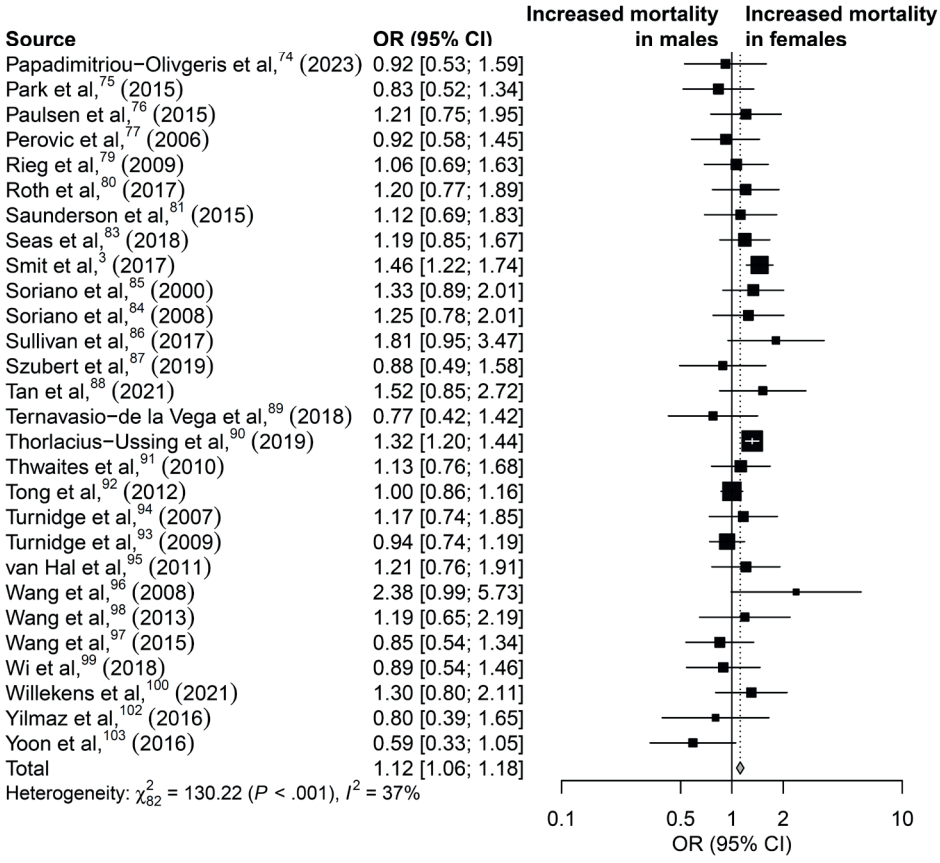


Figure 2 - continued



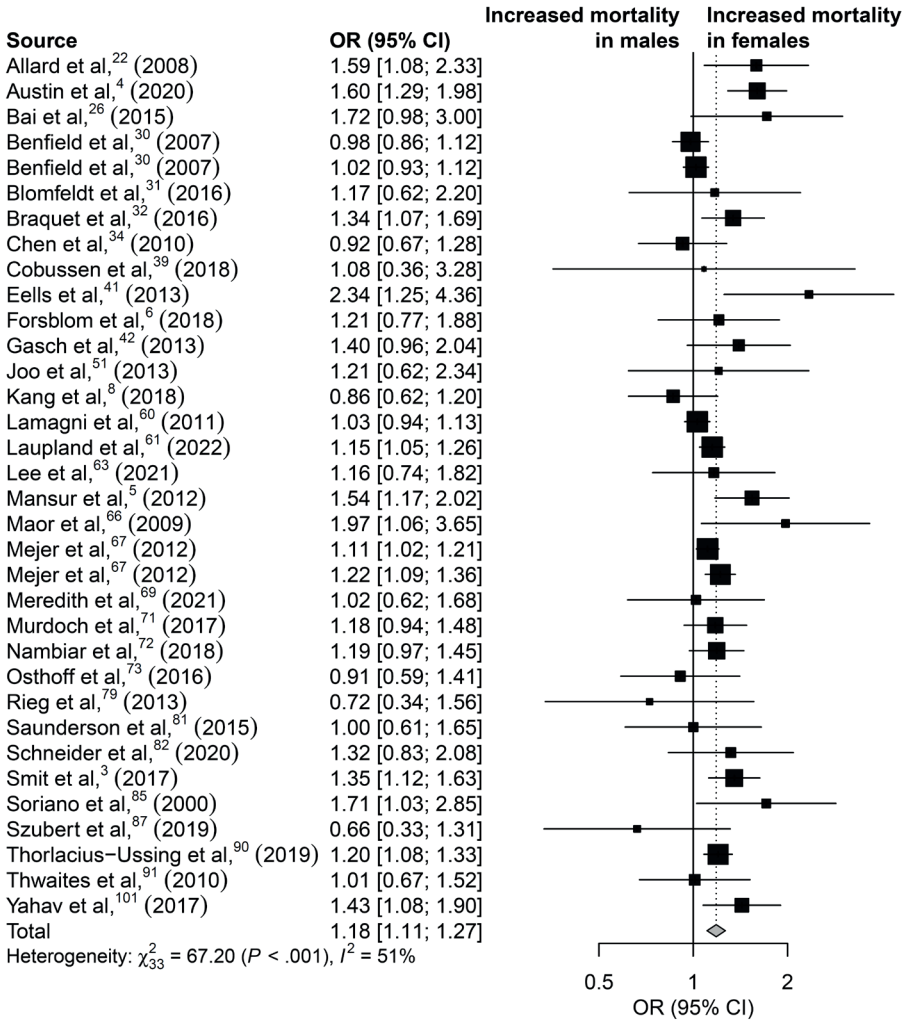
Adjusted mortality data that accounted for patient characteristics and treatment variables was available from 32 studies (95 469 patients) and revealed a similarly increased mortality risk in female relative to male patients (pooled adjusted OR [aOR], 1.18; 95% CI, 1.11-1.27) (Figure 3). An influence analysis revealed that exclusion of any single study did not significantly alter the findings from the overall cohort (eAppendix 5 in Supplement 1). A sensitivity analysis with only studies that had an OR that was either reported or could be directly calculated (ie, excluding 14 studies in which RR or HR were reported) similarly did not change the overall findings (eFigure 3 in Supplement 1). No funnel plot asymmetry was found (eFigure 4 in Supplement 1). Substantial heterogeneity was observed in this analysis of adjusted mortality data ($Q = 66.98$; $P < .001$; $I2 = 51\%$). Meta-analyses on subsets of studies showed that variation in the geographic location of the study impacted heterogeneity.

Meta-analyses of studies conducted in individual geographic regions all had lower observed heterogeneity than the overall cohort (overall $I2 = 51\%$): Europe (19 studies; $I2 = 41\%$), North America (5 studies; $I2 = 12\%$), East Asia (4 studies; $I2 = 0\%$), and Middle East (3 studies; $I2 = 0\%$). The pooled aOR varied significantly based on geographic location of study and ranged from 0.96 (95% CI, 0.76-1.22) for studies conducted in East Asia to 1.57 (95% CI, 1.23-2.01) for studies conducted in North America. Stratification of studies by mortality time point or by methicillin resistance did not impact heterogeneity.

Evaluation of the evidence

Given that this systematic review contained observational studies that accounted for confounding through statistical adjustment (ie, the adjusted analysis), the baseline strength of evidence was moderate. The mortality effect estimate was downrated due to a serious risk of bias because studies without a sex-difference in a univariable analysis would likely not have included this variable in a multivariable analysis. We did not have serious concerns about inconsistency, indirectness, imprecision, or publication bias. Therefore, the overall strength of evidence for the association of female sex with increased mortality risk in patients with SAB was low (eTable 2 in Supplement 1).

Figure 3. Forest plot of adjusted mortality in female vs male patients with *Staphylococcus aureus* bacteremia



Discussion

In this systematic review and meta-analysis, we addressed the question of whether female sex is associated with increased mortality risk in patients with SAB. The included studies involved over 130 000 patients and identified an association between female sex and increased mortality risk in both unadjusted and adjusted analyses. Heterogeneity was observed, but substantially decreased with stratification by geographic region. This may reflect the large practice variations for SAB throughout the world, as recently described in a global survey [102].

This study sheds new light on sex differences in clinical outcomes of patients with SAB, which is an area of little clarity. Few studies have primarily focused on sex differences in outcome in SAB patients, and their results have been contradictory. Some studies reported higher mortality in female patients with SAB compared with male patient [3,5], while others did not report an overall sex-difference in mortality [6,8]. In this meta-analysis we identified a relatively large (18%) increased odds of death in female patients compared with male patients. This association was significant in both the unadjusted analysis and in an adjusted analysis that accounted for patient co-morbidities and treatment variables. Beyond patients with SAB, excess mortality has been reported in female patients with hospital-acquired bloodstream infection [103], severe sepsis [104-106], and endocarditis [107]; however, conflicting evidence has been reported as well [108].

The underlying causes of sex differences in clinical outcomes of patients with SAB were not addressed in this study. Sex-related differences in outcome may be due to a variety of social or biological factors. Firm data for a biological connection between sex differences in clinical outcomes from animal models has been elusive. Previous studies on sepsis have generally supported better outcomes in female patients relative to male [109]. This has been hypothesized to stem from the positive immunomodulatory properties of sex hormones on cell-mediated immune responses and cardiovascular functions in female patients [110,111] as well as the suppression of the anti-infective response by testosterone in male patients [112]. Even an ongoing immunological advantage in postmenopausal septic women has been reported [113]. In *S aureus* infections in particular, an animal study showed enhanced neutrophil bactericidal capacity in female mice [114]. However, females were more susceptible to lethal toxic shock caused by *S aureus* enterotoxin B in another mouse model [115]. Social factors could also be contributing to the observed differences in mortality between female and male patients with SAB. Analogous to acute myocardial infarction, where women waited longer before seeking treatment relative to men, gender-differences in health seeking behavior may exist in SAB patients [116]. Gender bias in health care delivery can potentially contribute to the difference in

outcome as well. Delays in antibiotic treatment and less invasive treatment have been reported in women with septic shock and critical illness [105,117-119], and women were less likely to receive the recommended quality of acute care compared with men in a US study on quality of care in sociodemographic subgroups [120]. In a 2023 cohort study from our research group [121], women with SAB received shorter durations of antimicrobial treatment and were less likely to undergo transesophageal echocardiography compared to men. Regional or cultural differences in health care delivery could be impacting the observed sex-based difference in patient outcomes. The association between female sex and mortality varied to some degree by location of study, and we have previously shown that there is considerable global variation in SAB treatment factors [102]. Finally, response to treatment can differ between female and male patients. Both pharmacokinetics and pharmacodynamics are generally subject to sex influences [122].

Limitations

This study had several limitations. First, sex difference was not the primary outcome of interest in the majority of the included studies. Therefore, a number of studies did not include adjusted data for mortality by sex, and inclusion of this data could have influenced the results. Second, reporting bias can exist as studies may not report mortality stratified by sex if there was no significant difference in mortality. Third, heterogeneity exists not only in study methodology but also in the disease itself.

The clinical presentation of SAB may vary from uncomplicated intravenous catheter-related bacteremia to complicated metastatic disease. Because all studies on SAB patients were included in our study, sex-based differences in outcome could not be stratified by infection severity. Lastly, whether reported sex represented sex assigned at birth or gender, was often not specified.

Conclusions

In this systematic review and meta-analysis, observational cohort studies demonstrated an association between female sex and increased mortality risk in adult patients with SAB. This association remained significant after including only studies that adjusted for patient clinical and treatment variables. Future research should focus on understanding the underlying causes and on promoting better outcomes in female patients with SAB. Fundamental research on biological sex differences in immune response or pharmacology, examinations of sex-based differences in management of SAB, and better reporting of sex-specific outcomes in randomized clinical trials are necessary to better understand the observed sex-specific differences in mortality among patients with SAB.

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eTable 2. Evidence profile for association of female sex and mortality in patients with *Staphylococcus aureus* bacteremia

eAppendix 1. Search Strategy Report: Original Search

Topic: Association of female sex with mortality in patients with *Staphylococcus aureus* bloodstream infections

Searcher: SJK

Date: 10.31.2022 Updated 4.26.2023

Database (including vendor/platform): MEDLINE (via PubMed)

Set #	Search Strategy	Results
#1 Staph	"Staphylococcus aureus"[Mesh] OR "staphylococcus aureus"[tiab] OR "s. aureus"[tiab] OR "s aureus"[tiab] OR "staph aureus"[tiab]	**150579**
#2 Infection	"Endocarditis, Bacterial"[Mesh] OR "Bacteremia"[Mesh] OR bacteremia[tiab] OR bacteraemia[tiab] OR bacteremias[tiab] OR bacteraemias[tiab] OR bacteremic[tiab] OR bacteraemic[tiab] OR ((bloodstream[tiab] OR "blood stream"[tiab] OR bloodstreams[tiab] OR "blood streams"[tiab]) AND (infection[tiab] OR infections[tiab] OR infected[tiab] OR infect[tiab] OR infects[tiab] OR infecting[tiab])) OR endocarditis[tiab]	**105361**
#3 Mortality	"Mortality"[sh] OR "Mortality"[Mesh] OR mortality[tiab] OR mortalities[tiab] OR fatal[tiab] OR fatality[tiab] OR fatalities[tiab] OR death[tiab] OR deaths[tiab] OR dying[tiab] OR die[tiab] OR died[tiab]	**2467469**
#4 Sex	"Female"[Mesh] OR "Male"[Mesh] OR "Sex Factors"[Mesh] OR female[tiab] OR females[tiab] OR male[tiab] OR males[tiab] OR women[tiab] OR woman[tiab] OR "womens" OR "womans" OR men[tiab] OR gender[tiab] OR genders[tiab] OR sex[tiab] OR sexes[tiab]	**13302123**
#5	1 AND 2 AND 3 AND 4	**3106**
#6	AND ("2022/01/01"[Date - MeSH] : "3000"[Date - MeSH])	**119**
Validation String	27343816 OR 26873381 OR 30194636 OR 29667110 OR 31185081 OR 23141419	**6/6**

Database (including vendor/platform): Embase via Elsevier

Set #	Search Strategy	Results
#1 Staph	'Staphylococcus aureus'/exp OR 'staphylococcus aureus':ti,ab OR 's. aureus':ti,ab OR 's aureus':ti,ab OR 'staph aureus':ti,ab	**249714**
#2 Infection	'bacteremia'/exp OR 'bacterial endocarditis'/exp OR bacteremia:ti,ab OR bacteraemia:ti,ab OR bacteremias:ti,ab OR bacteraemias:ti,ab OR bacteremic:ti,ab OR bacteraemic:ti,ab OR ((bloodstream:ti,ab OR 'blood stream':ti,ab OR bloodstreams:ti,ab OR 'blood streams':ti,ab) AND (infection:ti,ab OR infections:ti,ab OR infected:ti,ab OR infect:ti,ab OR infects:ti,ab OR infecting:ti,ab)) OR endocarditis:ti,ab	**153847**

#3 Mortality	'mortality'/de OR 'mortality rate'/exp OR mortality:ti,ab OR mortalities:ti,ab OR fatal:ti,ab OR fatality:ti,ab OR fatalities:ti,ab OR death:ti,ab OR deaths:ti,ab OR dying:ti,ab OR die:ti,ab OR died:ti,ab	**3345439**
#4 Sex	'female'/exp OR 'male'/exp OR 'sex difference'/exp OR female:ti,ab OR females:ti,ab OR male:ti,ab OR males:ti,ab OR women:ti,ab OR woman:ti,ab OR womens OR womans OR men:ti,ab OR gender:ti,ab OR genders:ti,ab OR sex:ti,ab OR sexes:ti,ab	**16110857**
#5	#1 AND #2 AND #3 AND #4	**6105**
#6	#1 AND #2 AND #3 AND #4 AND [humans]/lim AND ([article]/lim OR [article in press]/lim OR [conference paper]/lim)	**4138**
#7	#1 AND #2 AND #3 AND #4 AND [humans]/lim AND ([article]/lim OR [article in press]/lim OR [conference paper]/lim) AND [01-09-2022]/sd NOT [27-04-2023]/sd	**334**

Database (including vendor/platform): Web of Science Core Collection (1900-present) via Clarivate

Set #	Search Strategy	Results
#1 Staph	TS=("staphylococcus aureus" OR "s. aureus" OR "s aureus" OR "staph aureus")	**179081**
#2 Infection	TS=(bacteremia OR bacteraemia OR bacteremias OR bacteraemias OR bacteremic OR bacteraemic OR ((bloodstream OR "blood stream" OR bloodstreams OR "blood streams") AND (infection OR infections OR infected OR infect OR infects OR infecting)) OR endocarditis)	**102399**
#3 Mortality	TS=(mortality OR mortalities OR fatal OR fatality OR fatalities OR death OR deaths OR dying OR die OR died)	**2964183**
#4 Sex	TS=(female OR females OR male OR males OR women OR woman OR womens OR womans OR men OR gender OR genders OR sex OR sexes)	**4924618**
#5	1 AND 2 AND 3 AND 4	**936**
#6	Refined by Publication Years: 2022 or 2023	**101**

eAppendix 2. Newcastle-Ottawa Quality Assessment Scale for assessing risk of bias in observational studies. Risk of bias was assessed with the Newcastle-Ottawa Assessment Scale using the questions below. The procedure for converting the responses to an overall risk of bias assessment (i.e., low, medium, or high risk of bias) is detailed here as well.

Selection

1. Representativeness of the exposed cohort

a. Truly representative of the average patient with *S. aureus* bloodstream infection in the community (*)

b. Somewhat representative of the average patient with *S. aureus* bloodstream infection in the community (*)

c. Selected group of patients

d. No description of the derivation of the cohort

2. Selection of the non-exposed cohort

a. Drawn from the same community as the exposed cohort (*)

b. Drawn from a different source

c. No description of the derivation of the non-exposed cohort

3. Ascertainment of exposure

a. Secure record (e.g. medical records) (*)

b. Structured interview (*)

c. Written self-report

d. No description

4. Demonstration that outcome of interest was not present at start of study

a. Yes (*)

b. No

Comparability of cohorts on basis of design or analysis

1. Study controls for level of acute illness

a. Yes (*)

b. No

2. Study controls for any additional factor.

a. Yes (*)

b. No

Outcome

1. Assessment of outcome

a. Independent blind assessment (*)

b. Record linkage (*)

c. Self-report

d. No description

2. Was follow-up long enough for outcomes to occur

a. Yes (*)

b. No

3. Adequacy of follow up of cohorts

a. Complete follow up (all subjects accounted for) (*)

b. Subjects lost to follow up unlikely to introduce bias (≤10% lost to follow-up, or description provided of those lost) (*)

c. Follow up rate < 90% and no description of those lost

d. No statement

Thresholds used to convert the Newcastle-Ottawa scale to categories (good, fair, and poor):

Good quality/low risk of bias: 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain

Fair quality/medium risk of bias: 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain.

Poor quality/high risk of bias: 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain

eAppendix 3. Description of EPC approach.

We used the Evidence-based Practice Center (EPC) model from the U.S. Agency for Healthcare Research and Quality (AHRQ) to grade the overall strength of evidence [20]. The EPC approach evaluates the following domains: study limitations/risk of bias, consistency, directness, precision, and reporting bias. In brief, the EPC classification system applies an overall strength of evidence grade rating to an estimate effect from a body of evidence: high (we are very confident that the estimate of effect lies close to the true effect for this outcome), moderate (we are moderately confident that the estimate of effect lies close to the true effect for this outcome), low (we have limited confidence that the estimate of effect lies close to the true effect for this outcome), or insufficient (we have no evidence, we are unable to estimate an effect, or we have no confidence in the estimate of effect for this outcome). The initial strength of evidence grade was moderate given that the included observational studies in the primary adjusted analysis reduced bias from confounding through matching or statistical adjustment [20]. This baseline category could be rated down if the included studies demonstrated high risk of bias, imprecision, inconsistency, indirectness, or reporting bias.

eTable 1 (next page). Newcastle-Ottawa quality assessment of individual studies
The Newcastle-Ottawa Quality Assessment Scale determines a study's risk of bias through nine questions (detailed in Appendix 2). For each study, the grades for the nine questions are shown below. Grades that receive a star are highlighted in green, while those that do not are highlighted in red. Based on the grades from each question in the Newcastle-Ottawa Scale, an overall risk of bias (high, medium, low) can be assigned (detailed in Appendix 2).

Study	**Selection:** Representativeness of the exposed cohort	**Selection:** Selection of the non-exposed cohort	**Selection:** Ascertainment of exposure	**Selection:** Outcome of interest not present at start	**Comparability:** Study controls for level of acute illness	**Comparability:** Study controls for any additional factor	**Outcome:** Assessment of outcome	**Outcome:** Follow-up long enough for outcomes to occur	**Outcome:** Adequacy of follow up of cohorts	**Risk of bias**
Abbas 2020	b	a	a	a	b	b	b	a	a	Poor
Allard 2008	b	a	a	a	b	a	b	a	a	Good
Ammerlaan 2009	a	a	a	a	b	b	b	a	a	Poor
Asgeirsson 2011	b	a	a	a	b	b	b	a	a	Poor
Austin 2020	b	a	a	a	a	a	b	a	a	Good
Ayau 2017	c	a	a	a	b	b	b	a	a	Poor
Bai 2015	c	a	a	a	a	a	b	a	a	Good
Bassetti 2017	b	a	a	a	b	b	b	a	a	Poor
Battle 2022	b	a	a	a	b	b	b	a	a	Poor
Ben-Zvi 2019	b	a	a	a	b	b	b	a	a	Poor
Benfield 2007	b	a	a	a	b	a	b	a	b	Good
Blomfeldt 2016	b	a	a	a	b	a	b	a	d	Good
Braquet 2016	b	a	a	a	a	a	b	a	b	Good
Chavez 2022	b	a	a	a	b	b	b	a	d	Poor
Chen 2010	b	a	a	a	a	a	b	a	a	Good
Chen 2015	b	a	a	a	b	b	b	a	b	Poor
Chen 2021	c	a	a	a	b	b	b	a	a	Poor
Chihara 2009	c	a	a	a	b	b	b	a	a	Poor
Chung 2021	c	a	a	a	b	b	b	a	a	Poor
Cobussen 2018	b	a	a	a	a	a	b	a	a	Good

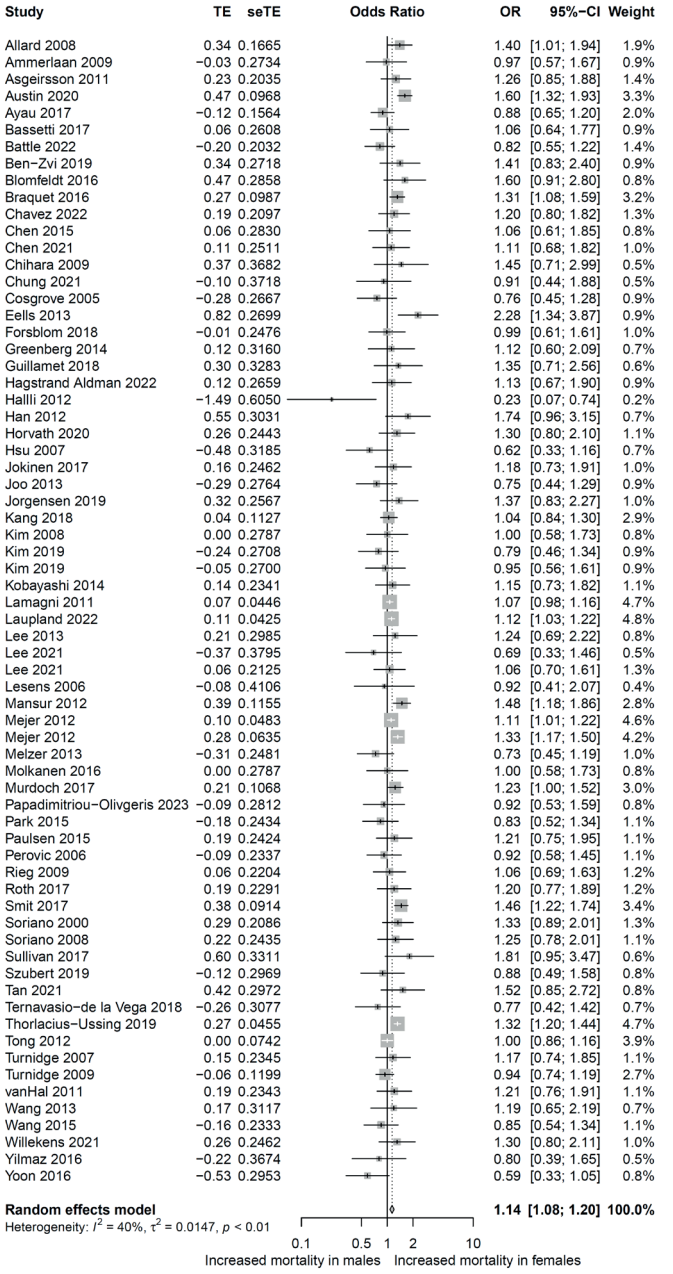
Cosgrove 2005	b	a	a	a	b	b	b	a	a	Poor
Eells 2013	c	a	a	a	a	a	b	a	a	Good
Forsblom 2018	c	a	a	a	a	a	b	a	b	Good
Gasch 2013	c	a	a	a	a	a	b	a	b	Good
Greenberg 2014	b	a	a	a	b	b	b	a	a	Poor
Guillamet 2018	c	a	a	a	b	b	b	a	b	Poor
HagstrandAldman 2022	c	a	a	a	b	b	b	a	a	Poor
Hallli 2012	c	a	a	a	b	b	b	a	a	Poor
Han 2012	b	a	a	a	b	b	b	a	a	Poor
Honda 2010	b	a	a	a	b	b	b	a	a	Poor
Horváth 2020	b	a	a	a	b	b	b	a	a	Poor
Hsu 2007	c	a	a	a	b	b	b	a	a	Poor
Jokinen 2017	b	a	a	a	b	b	b	a	c	Poor
Joo 2013	c	a	a	a	a	a	b	a	a	Good
Joost 2017	b	a	a	a	b	b	b	a	d	Poor
Jorgensen 2019	c	a	a	a	b	b	b	a	a	Poor
Kang 2018	b	a	a	a	a	a	b	a	d	Good
Kempker 2010	c	a	a	a	b	b	b	a	a	Poor
Kim 2008	b	a	a	a	b	b	b	a	a	Poor
Kim 2010	b	a	a	a	b	b	b	a	a	Poor
Kim 2019	b	a	a	a	b	b	b	a	d	Poor
Kim 2019	b	a	a	a	b	b	b	a	a	Poor
Kobayashi 2014	b	a	a	a	b	b	b	a	a	Poor
Lamagni 2011	c	a	a	a	a	a	b	a	a	Good
Laupland 2022	b	a	a	a	a	a	b	a	d	Good
Lee 2013	c	a	a	a	b	b	b	a	a	Poor
Lee 2021	c	a	a	a	b	a	b	a	a	Good
Lee 2021	c	a	a	a	b	b	b	a	a	Poor
Lesens 2006	a	a	a	a	b	b	b	a	a	Poor
Mansur 2012	b	a	a	a	a	a	b	a	a	Good
Maor 2009	c	a	a	a	b	a	b	a	d	Good
Mejer 2012	b	a	a	a	b	a	b	a	b	Good
Melzer 2013	c	a	a	a	b	b	b	a	a	Poor
Meredith 2021	b	a	a	a	a	a	b	a	d	Good
Mölkänen 2016	b	a	a	a	b	b	b	a	d	Poor
Murdoch 2017	b	a	a	a	b	a	b	a	d	Good

Nambiar 2018	a	a	a	a	a	a	b	a	d	Good
Osthoff 2016	b	a	a	a	b	a	b	a	d	Good
Papadimitriou-Olivgeris	b	a	a	a	b	b	b	a	d	Poor
Park 2015	b	a	a	a	b	b	b	a	d	Poor
Paulsen 2015	b	a	a	a	b	b	b	a	a	Poor
Perovic 2006	b	a	a	a	b	b	b	a	b	Poor
Rieg 2009	b	a	a	a	b	b	b	a	a	Poor
Rieg 2013	b	a	a	a	b	a	b	a	d	Good
Roth 2017	b	a	a	a	b	b	b	a	b	Poor
Saunderson 2015	b	a	a	a	b	a	b	a	a	Good
Schneider 2020	b	a	a	a	b	a	b	a	a	Good
Seas 2018	a	a	a	a	b	b	b	a	b	Poor
Smit 2017	b	a	a	a	a	a	b	a	a	Good
Soriano 2000	b	a	a	a	a	a	b	a	a	Good
Soriano 2008	c	a	a	a	b	b	b	a	a	Poor
Sullivan 2017	c	a	a	a	b	b	b	a	d	Poor
Szubert 2019	b	a	a	a	a	a	b	a	d	Good
Tan 2021	b	a	a	a	b	b	b	a	d	Poor
Ternavasio-delaVega 2018	b	a	a	a	b	b	b	a	a	Poor
Thorlacius-Ussing 2019	b	a	a	a	b	a	b	a	a	Good
Thwaites 2010	a	a	a	a	b	a	b	a	b	Good
Tong 2012	b	a	a	a	b	b	b	a	a	Poor
Turnidge 2007	b	a	a	a	b	b	b	a	c	Poor
Turnidge 2009	b	a	a	a	b	b	b	a	a	Poor
vanHal 2011	b	a	a	a	b	b	b	a	a	Poor
Wang 2008	c	a	a	a	b	b	b	a	a	Poor
Wang 2013	b	c	a	a	b	b	b	a	a	Poor
Wang 2015	c	a	a	a	b	b	b	a	a	Poor
Wi 2018	a	c	a	a	b	b	b	a	d	Poor
Willekens 2021	b	a	a	a	b	b	b	a	a	Poor
Yahav 2017	b	c	a	a	b	a	b	a	a	Good
Yilmaz 2016	b	a	a	a	b	b	b	a	d	Poor
Yoon 2016	b	a	a	a	b	b	b	a	d	Poor

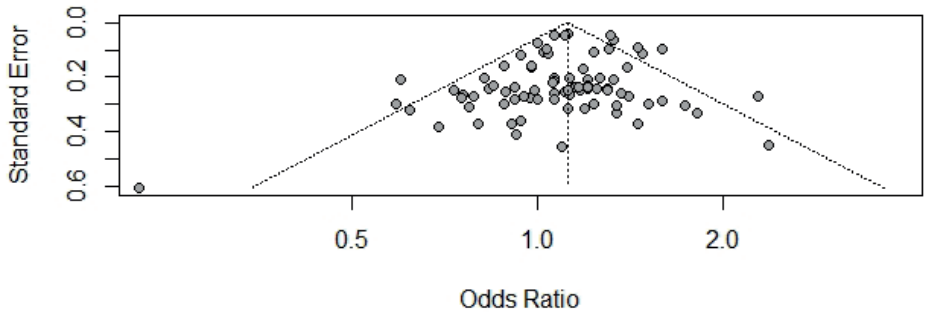
eAppendix 4. Influence analysis of unadjusted mortality in patients with *Staphylococcus aureus* bacteremia. An influence analysis showed that the overall results of the meta-analysis (i.e., association of female sex with increased mortality) did not change with removal of individual studies.

	OR	95%-CI	p-value	tau^2	tau	I^2
Omitting Abbas 2020	1.1193	[1.0648; 1.1766]	< 0.0001	0.0139	0.1178	37.8%
Omitting Abbas 2020	1.1201	[1.0656; 1.1774]	< 0.0001	0.0138	0.1177	37.6%
Omitting Allard 2008	1.1153	[1.0611; 1.1724]	< 0.0001	0.0139	0.1178	37.0%
Omitting Ammerlaan 2009	1.1203	[1.0657; 1.1777]	< 0.0001	0.0139	0.1179	37.6%
Omitting Asgeirsson 2011	1.1174	[1.0627; 1.1748]	< 0.0001	0.0141	0.1189	37.6%
Omitting Austin 2020	1.1119	[1.0605; 1.1659]	< 0.0001	0.0101	0.1007	31.3%
Omitting Ayau 2017	1.1242	[1.0697; 1.1814]	< 0.0001	0.0133	0.1153	36.4%
Omitting Bassetti 2017	1.1195	[1.0648; 1.1770]	< 0.0001	0.0140	0.1183	37.7%
Omitting Battle 2022	1.1238	[1.0696; 1.1807]	< 0.0001	0.0133	0.1153	36.4%
Omitting Ben-Zvi 2019	1.1172	[1.0629; 1.1744]	< 0.0001	0.0140	0.1182	37.5%
Omitting Blomfeldt 2016	1.1166	[1.0625; 1.1733]	< 0.0001	0.0138	0.1177	37.1%
Omitting Braquet 2016	1.1143	[1.0597; 1.1717]	< 0.0001	0.0139	0.1181	36.8%
Omitting Chavez 2022	1.1180	[1.0633; 1.1755]	< 0.0001	0.0142	0.1190	37.7%
Omitting Chen 2010	1.1215	[1.0667; 1.1791]	< 0.0001	0.0139	0.1180	37.3%
Omitting Chen 2015	1.1195	[1.0648; 1.1769]	< 0.0001	0.0140	0.1182	37.7%
Omitting Chen 2021	1.1191	[1.0644; 1.1766]	< 0.0001	0.0140	0.1185	37.8%
Omitting Chihara 2009	1.1179	[1.0636; 1.1750]	< 0.0001	0.0139	0.1180	37.6%
Omitting Chung 2021	1.1202	[1.0657; 1.1775]	< 0.0001	0.0138	0.1176	37.6%
Omitting Cosgrove 2005	1.1229	[1.0688; 1.1797]	< 0.0001	0.0134	0.1159	36.6%
Omitting Eells 2013	1.1144	[1.0622; 1.1691]	< 0.0001	0.0130	0.1140	34.5%
Omitting Forsblom 2018	1.1203	[1.0656; 1.1778]	< 0.0001	0.0139	0.1181	37.6%
Omitting Gasch 2013	1.1181	[1.0633; 1.1757]	< 0.0001	0.0142	0.1192	37.7%
Omitting Greenberg 2014	1.1191	[1.0645; 1.1765]	< 0.0001	0.0140	0.1182	37.8%
Omitting Guillamet 2018	1.1180	[1.0636; 1.1753]	< 0.0001	0.0140	0.1181	37.6%
Omitting Hagstrand Aldman 2022	1.1190	[1.0643; 1.1764]	< 0.0001	0.0140	0.1184	37.8%
Omitting HallIi 2012	1.1227	[1.0705; 1.1775]	< 0.0001	0.0133	0.1155	34.1%
Omitting Han 2012	1.1163	[1.0625; 1.1729]	< 0.0001	0.0138	0.1174	36.8%
Omitting Honda 2010	1.1181	[1.0635; 1.1754]	< 0.0001	0.0140	0.1183	37.7%
Omitting Horvath 2020	1.1176	[1.0630; 1.1749]	< 0.0001	0.0141	0.1185	37.6%
Omitting Hsu 2007	1.1234	[1.0698; 1.1797]	< 0.0001	0.0132	0.1151	35.9%
Omitting Jokinen 2017	1.1185	[1.0638; 1.1760]	< 0.0001	0.0141	0.1186	37.8%
Omitting Joo 2013	1.1227	[1.0687; 1.1795]	< 0.0001	0.0134	0.1160	36.6%
Omitting Joost 2017	1.1214	[1.0662; 1.1795]	< 0.0001	0.0142	0.1191	37.2%
Omitting Jorgensen 2019	1.1172	[1.0628; 1.1744]	< 0.0001	0.0140	0.1183	37.5%
Omitting Kang 2018	1.1207	[1.0655; 1.1787]	< 0.0001	0.0143	0.1196	37.4%
Omitting Kempker 2010	1.1216	[1.0668; 1.1793]	< 0.0001	0.0139	0.1180	37.3%
Omitting Kim 2008	1.1200	[1.0654; 1.1774]	< 0.0001	0.0139	0.1180	37.6%
Omitting Kim 2010	1.1196	[1.0648; 1.1772]	< 0.0001	0.0141	0.1188	37.7%
Omitting Kim 2019	1.1224	[1.0682; 1.1793]	< 0.0001	0.0135	0.1163	36.8%
Omitting Kim 2019	1.1205	[1.0659; 1.1779]	< 0.0001	0.0139	0.1178	37.5%
Omitting Kobayashi 2014	1.1187	[1.0640; 1.1762]	< 0.0001	0.0141	0.1187	37.8%
Omitting Lamagni 2011	1.1204	[1.0646; 1.1791]	< 0.0001	0.0148	0.1215	36.4%
Omitting Laupland 2022	1.1179	[1.0621; 1.1766]	< 0.0001	0.0151	0.1228	37.6%
Omitting Lee 2013	1.1184	[1.0638; 1.1757]	< 0.0001	0.0140	0.1183	37.7%
Omitting Lee 2021	1.1216	[1.0675; 1.1784]	< 0.0001	0.0136	0.1167	36.9%
Omitting Lee 2021	1.1196	[1.0648; 1.1772]	< 0.0001	0.0141	0.1187	37.7%
Omitting Lesens 2006	1.1200	[1.0655; 1.1772]	< 0.0001	0.0138	0.1176	37.6%
Omitting Mansur 2012	1.1130	[1.0596; 1.1691]	< 0.0001	0.0127	0.1127	35.3%
Omitting Mejer 2012	1.1185	[1.0628; 1.1772]	< 0.0001	0.0150	0.1225	37.5%
Omitting Mejer 2012	1.1128	[1.0585; 1.1699]	< 0.0001	0.0133	0.1152	34.9%
Omitting Melzer 2013	1.1239	[1.0700; 1.1805]	< 0.0001	0.0132	0.1149	36.1%
Omitting Molkanen 2016	1.1200	[1.0654; 1.1774]	< 0.0001	0.0139	0.1180	37.6%
Omitting Murdoch 2017	1.1158	[1.0609; 1.1737]	< 0.0001	0.0144	0.1201	37.5%
Omitting Nambiar 2018	1.1212	[1.0659; 1.1793]	< 0.0001	0.0143	0.1196	37.2%
Omitting Osthoff 2016	1.1292	[1.0770; 1.1838]	< 0.0001	0.0116	0.1075	32.8%
Omitting Papadimitriou-Olivgeris 2023	1.1208	[1.0662; 1.1781]	< 0.0001	0.0138	0.1176	37.5%
Omitting Park 2015	1.1224	[1.0680; 1.1794]	< 0.0001	0.0136	0.1165	36.9%
Omitting Paulsen 2015	1.1182	[1.0636; 1.1757]	< 0.0001	0.0141	0.1187	37.7%
Omitting Perovic 2006	1.1213	[1.0668; 1.1787]	< 0.0001	0.0138	0.1175	37.3%
Omitting Rieg 2009	1.1196	[1.0648; 1.1772]	< 0.0001	0.0141	0.1186	37.7%
Omitting Roth 2017	1.1182	[1.0635; 1.1757]	< 0.0001	0.0141	0.1188	37.7%
Omitting Saunderson 2015	1.1190	[1.0643; 1.1765]	< 0.0001	0.0141	0.1186	37.8%
Omitting Seas 2018	1.1178	[1.0629; 1.1754]	< 0.0001	0.0143	0.1195	37.7%
Omitting Smit 2017	1.1124	[1.0591; 1.1682]	< 0.0001	0.0121	0.1101	34.2%
Omitting Soriano 2000	1.1168	[1.0623; 1.1741]	< 0.0001	0.0141	0.1186	37.5%
Omitting Soriano 2008	1.1179	[1.0633; 1.1753]	< 0.0001	0.0141	0.1186	37.7%
Omitting Sullivan 2017	1.1165	[1.0627; 1.1730]	< 0.0001	0.0138	0.1174	36.8%
Omitting Szubert 2019	1.1209	[1.0665; 1.1782]	< 0.0001	0.0138	0.1174	37.4%
Omitting Tan 2021	1.1170	[1.0628; 1.1740]	< 0.0001	0.0139	0.1179	37.3%
Omitting Ternavasio-de la Vega 2018	1.1219	[1.0676; 1.1788]	< 0.0001	0.0136	0.1167	37.0%
Omitting Thorlacius-Ussing 2019	1.1124	[1.0580; 1.1696]	< 0.0001	0.0131	0.1146	32.4%
Omitting Thwaites 2010	1.1187	[1.0639; 1.1763]	< 0.0001	0.0142	0.1190	37.8%
Omitting Tong 2012	1.1234	[1.0681; 1.1816]	< 0.0001	0.0139	0.1178	36.0%
Omitting Turnidge 2007	1.1185	[1.0638; 1.1761]	< 0.0001	0.0141	0.1187	37.8%
Omitting Turnidge 2009	1.1242	[1.0695; 1.1818]	< 0.0001	0.0134	0.1158	36.4%
Omitting vanHal 2011	1.1182	[1.0635; 1.1757]	< 0.0001	0.0141	0.1187	37.7%
Omitting Wang 2008	1.1168	[1.0634; 1.1730]	< 0.0001	0.0138	0.1173	36.5%
Omitting Wang 2013	1.1187	[1.0641; 1.1761]	< 0.0001	0.0140	0.1182	37.8%
Omitting Wang 2015	1.1224	[1.0680; 1.1795]	< 0.0001	0.0136	0.1166	37.0%
Omitting Wi 2018	1.1214	[1.0669; 1.1787]	< 0.0001	0.0138	0.1173	37.3%
Omitting Willekens 2021	1.1176	[1.0630; 1.1749]	< 0.0001	0.0140	0.1185	37.6%
Omitting Yilmaz 2016	1.1209	[1.0666; 1.1780]	< 0.0001	0.0137	0.1172	37.3%
Omitting Yoon 2016	1.1246	[1.0714; 1.1804]	< 0.0001	0.0130	0.1138	35.2%
Pooled estimate	1.1193	[1.0652; 1.1761]	< 0.0001	0.0138	0.1174	37.0%

eFigure 1. Sensitivity analysis of unadjusted mortality. Only studies that either directly reported an odds ratio (OR) or contained raw mortality data such that ORs could be directly calculated are included here. Studies that reported a hazard ratio, relative risk, or mortality rate ratio were excluded.



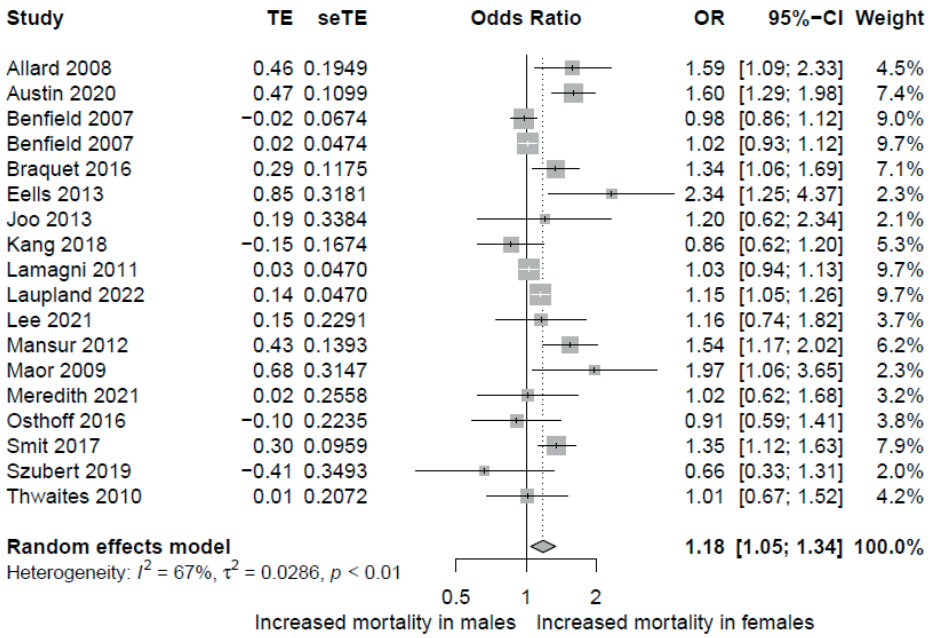
eFigure 2. Funnel plot of studies included in the analysis of unadjusted mortality. One study in particular had an effect size that was larger than expected based on the standard error (lower left corner of plot). This study demonstrated significantly lower mortality in females relative to males. Despite this, Egger's test did not reveal significant asymmetry in the funnel plot (p=0.06). Thus in total no clear evidence of publication bias was detected.



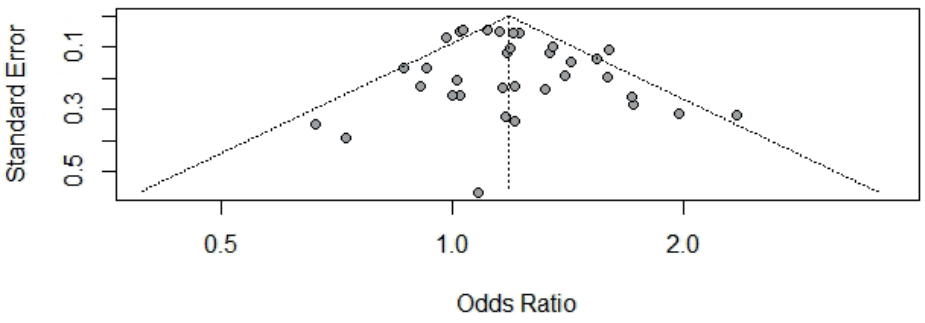
eAppendix 5. Influence analysis of adjusted mortality in patients with *Staphylococcus aureus* bacteremia. An influence analysis showed that the overall results of the meta-analysis (i.e., association of female sex with increased mortality) did not change with removal of individual studies.

	OR	95%-CI	p-value	tau^2	tau	I^2
Omitting Allard 2008	1.1757	[1.0998; 1.2568]	< 0.0001	0.0117	0.1084	50.0%
Omitting Austin 2020	1.1619	[1.0920; 1.2363]	< 0.0001	0.0081	0.0898	44.1%
Omitting Bai 2015	1.1784	[1.1021; 1.2600]	< 0.0001	0.0121	0.1102	50.7%
Omitting Benfield 2007	1.1956	[1.1181; 1.2785]	< 0.0001	0.0109	0.1045	48.1%
Omitting Benfield 2007	1.1956	[1.1164; 1.2805]	< 0.0001	0.0117	0.1084	47.4%
Omitting Blomfeldt 2016	1.1840	[1.1053; 1.2683]	< 0.0001	0.0128	0.1132	52.2%
Omitting Braquet 2016	1.1780	[1.0993; 1.2623]	< 0.0001	0.0127	0.1127	50.8%
Omitting Chen 2010	1.1911	[1.1132; 1.2744]	< 0.0001	0.0125	0.1119	51.1%
Omitting Cobussen 2018	1.1840	[1.1057; 1.2678]	< 0.0001	0.0126	0.1124	52.2%
Omitting Eells 2013	1.1753	[1.1026; 1.2528]	< 0.0001	0.0114	0.1069	48.3%
Omitting Forsblom 2018	1.1838	[1.1047; 1.2685]	< 0.0001	0.0130	0.1140	52.2%
Omitting Gasch 2013	1.1796	[1.1016; 1.2632]	< 0.0001	0.0126	0.1123	51.4%
Omitting Joo 2013	1.1837	[1.1051; 1.2678]	< 0.0001	0.0128	0.1130	52.2%
Omitting Kang 2018	1.1923	[1.1158; 1.2740]	< 0.0001	0.0120	0.1094	50.1%
Omitting Lamagni 2011	1.1953	[1.1155; 1.2807]	< 0.0001	0.0122	0.1103	48.1%
Omitting Laupland 2022	1.1884	[1.1061; 1.2769]	< 0.0001	0.0146	0.1209	52.2%
Omitting Lee 2021	1.1845	[1.1054; 1.2693]	< 0.0001	0.0130	0.1142	52.2%
Omitting Mansur 2012	1.1715	[1.0967; 1.2514]	< 0.0001	0.0108	0.1041	48.6%
Omitting Maor 2009	1.1775	[1.1024; 1.2577]	< 0.0001	0.0119	0.1092	50.0%
Omitting Mejer 2012	1.1911	[1.1089; 1.2793]	< 0.0001	0.0143	0.1196	51.9%
Omitting Mejer 2012	1.1833	[1.1016; 1.2711]	< 0.0001	0.0143	0.1196	51.0%
Omitting Meredith 2021	1.1864	[1.1076; 1.2708]	< 0.0001	0.0129	0.1138	52.1%
Omitting Murdoch 2017	1.1853	[1.1047; 1.2718]	< 0.0001	0.0138	0.1174	52.2%
Omitting Nambiar 2018	1.1850	[1.1042; 1.2718]	< 0.0001	0.0139	0.1180	52.1%
Omitting Osthoff 2016	1.1890	[1.1110; 1.2725]	< 0.0001	0.0128	0.1129	51.5%
Omitting Rieg 2013	1.1873	[1.1102; 1.2697]	< 0.0001	0.0126	0.1124	51.2%
Omitting Saunderson 2015	1.1867	[1.1080; 1.2710]	< 0.0001	0.0129	0.1137	52.0%
Omitting Schneider 2020	1.1820	[1.1034; 1.2661]	< 0.0001	0.0128	0.1132	51.9%
Omitting Smit 2017	1.1757	[1.0974; 1.2596]	< 0.0001	0.0123	0.1110	49.8%
Omitting Soriano 2000	1.1776	[1.1015; 1.2588]	< 0.0001	0.0120	0.1096	50.4%
Omitting Szubert 2019	1.1887	[1.1127; 1.2698]	< 0.0001	0.0125	0.1117	50.4%
Omitting Thorlacius-Ussing 2019	1.1848	[1.1028; 1.2729]	< 0.0001	0.0145	0.1204	51.5%
Omitting Thwaites 2010	1.1876	[1.1087; 1.2722]	< 0.0001	0.0130	0.1142	52.0%
Omitting Yahav 2017	1.1762	[1.0989; 1.2589]	< 0.0001	0.0121	0.1100	50.4%
Pooled estimate	1.1836	[1.1067; 1.2658]	< 0.0001	0.0125	0.1119	50.7%

eFigure 3. Sensitivity analysis adjusted mortality. Only studies that either directly reported an odds ratio (OR) or contained raw mortality data such that ORs could be directly calculated are included here. Studies that reported a hazard ratio, relative risk, or mortality rate ratio were excluded.

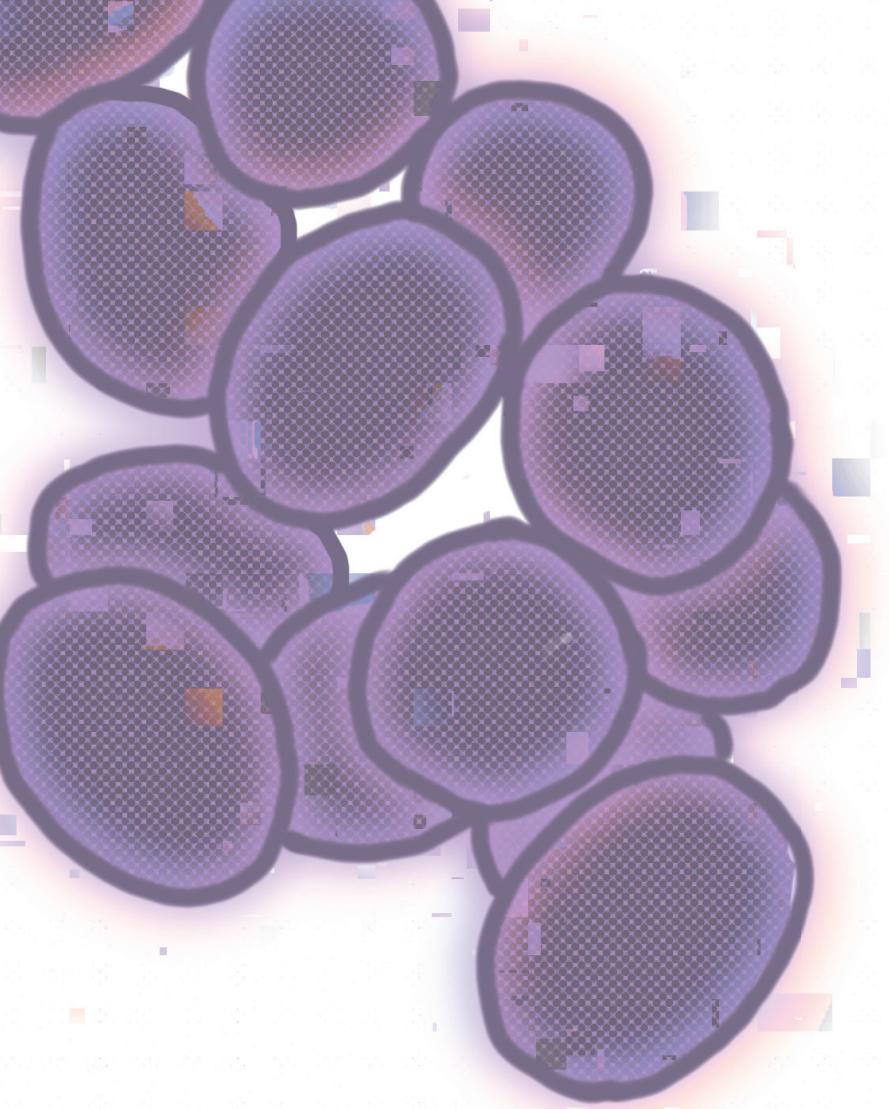


eFigure 4. Funnel plot for studies included in analysis of adjusted mortality. Egger's test did not reveal significant asymmetry in the funnel plot (p=0.10).



eTable 2. Evidence profile for association of female sex and mortality in patients with *Staphylococcus aureus* bacteremia.

Patient population	Patients with *Staphylococcus aureus* bacteremia
Setting	Hospital
Intervention	Female sex
Comparison	Male sex
Outcome	Mortality
Studies (participants)	89 (132,582)
Risk of bias	High
Consistency	Consistent
Precision	Precise
Directness	Direct
Other limitations	Sex-difference was not the primary outcome of interest in the majority of the studies that were included
Overall strength of evidence	Low
Conclusion	Female sex is associated with higher mortality in patients with SAB
Summary estimate	Unadjusted: 1.12 (95%CI 1.07-1.18) Adjusted: 1.18 (95%CI 1.11-1.27)



Chapter 11

Summary and general discussion

Summary and general discussion

Staphylococcus aureus colonizes millions of people, often without causing any symptoms. In contrast, when mucosal or skin barriers are broken, *S. aureus* becomes a frequent cause of hospital-acquired, healthcare-associated, and community-acquired infections in all age categories. *S. aureus* disease is highly variable, ranging from mild skin infections to catastrophic bloodstream infections with high mortality rates. Perhaps as a result of this heterogeneity, many questions remain with respect to risk factors, complications, and management.

The resistant variant of *S. aureus* is a major threat to global public health. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a dominant actor in antimicrobial resistance. MRSA colonization increases infection risks, forming the basis for decolonization of MRSA carriers. This thesis addressed the optimization of MRSA decolonization strategies and frequently encountered challenges in *S. aureus* bacteremia management. The results of the studies described in chapters 2 through 10 will be briefly summarized and discussed in this chapter.

Optimization of MRSA decolonization

In the Netherlands, we are proud of having one of the world's lowest rates of MRSA. Less than 5% of invasive *S. aureus* isolates in our country are resistant to methicillin, compared to up to 25% in our neighboring countries [1]. Yet, given the rising MRSA prevalence in our surrounding countries, the immigration of people from high-endemic areas, and the travelling of Dutch citizens towards these regions, it requires our continuous attention. The 'search and destroy' policy targeting MRSA is executed in the Netherlands since 1988 and has since been proven to be cost-effective [2, 3].

However, the effectiveness of the 'search and destroy' policy as a whole, depends on several consecutive steps. Analogous to the renowned cascade of care for persons living with HIV, that has been frequently used to identify culprits in the uptake of antiretroviral therapy [4, 5], we constructed a cascade of care for MRSA decolonization. Each consecutive step of this conceptual cascade is crucial, since individuals may be lost in every step. The first steps include identification of carriers and the initiation of treatment, and were analyzed in **chapter 2**. We surveyed 114 general practitioners about their familiarity with the 'search and destroy' policy and evaluated barriers in the uptake of MRSA eradication care. Remarkably, the majority of the responding general practitioners were not familiar with the policy. Moreover, they often refrained from starting eradication treatment, for various reasons including lack of recommendation in a general practitioners' guideline, patients' burden and

out-of-pocket costs. The most apparent improvements in these steps therefore lie in expanding familiarity with the 'search and destroy' policy and incorporating it in a general practitioners' guideline. In addition, treatment initiation should be made as accessible as possible, for example by facilitating easy referrals and eliminating costs for the individual patient.

It is essential to realize that the aforementioned study focuses specifically on the Dutch situation and is not necessarily applicable to the rest of the world. MRSA endemicity varies widely around the globe, significantly impacting the rationale behind decolonization treatments, as described in **chapter 3**. Due to the high risk of recolonization in the setting of high MRSA prevalence in the community, the likelihood of successful long-term decolonization is low. In this setting, a standard 'search and destroy' policy is not likely to attribute to lowering its prevalence in the population as a whole. Short-term bacterial load reduction aiming at prevention of nosocomial infections and transmission might be appropriate in countries where MRSA is endemic. Nevertheless, a broader approach with nationwide infection control programs is able to reduce the high prevalence of MRSA in healthcare settings drastically, as demonstrated in the United Kingdom at the beginning of this century [6]. Furthermore, individual risk factors for treatment failure contribute to likelihood of successful eradication. Thus, both likelihood of successful durable eradication and treatment goal should guide the eligibility for community-onset MRSA decolonization treatment of the individual patient.

The last step in the MRSA cascade of care concerns the effectiveness of decolonization treatments. In **chapter 3**, we describe the effectivity of different decolonization treatments. The combination of mupirocin and antiseptic body wash is highly effective in decolonization of nasal MRSA carriage but appears to be insufficient in patients with extra-nasal MRSA colonization. Most evidence supports topical therapy combined with rifampin and a second antimicrobial agent for extra-nasal MRSA eradication. However, the clinical applicability of many studies on MRSA decolonization is hampered by the lack of reporting of the carrier status of household contacts and long-term follow-up cultures. Also, the MRSA colonization rate in the population varies between studies and is believed to be a major driver of recolonization. In this respect, it is of importance that strain genotype is often not reported in case of positive follow up cultures, which makes differentiating between treatment failure and recolonization impossible. Future studies should include these factors, to accurately determine the most effective treatment and the real risk of recolonization in low and high prevalent settings.

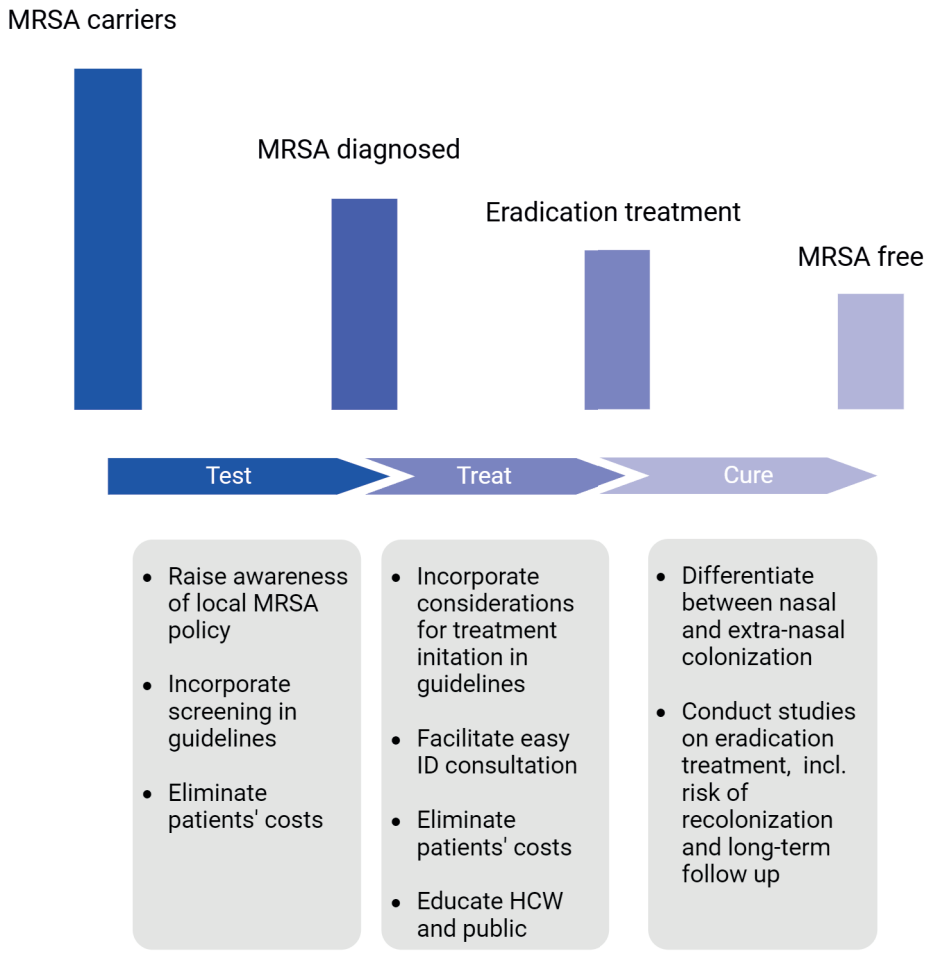
In order to provide insight in the situation in our region, we evaluated the efficacy of decolonization treatments in complicated MRSA carriership in five Dutch hospitals in **chapter 4**. We found an overall high success rate, and a trend towards a higher

success rate in patients treated with oral rifampin and doxycycline. Due to the retrospective design of the study and the small sample size, the causal relationship of this antimicrobial regimen with the higher success rate is not yet indisputably proven. To evaluate this further, we are currently conducting the CLEANEST study, a multicenter cluster-randomized trial comparing rifampin-doxycycline with rifampin-trimethoprim for the treatment of complicated MRSA colonization.

Apart from recolonization risks and individual risk factors for treatment failure, differences in genetic characteristics of the MRSA isolates may play a role in the probability of successful eradication. In **chapter 5**, we performed an explorative study on genetic determinants of MRSA isolates and their association with decolonization treatment outcome. We found a higher eradication failure rate in complicated MRSA carriers with ciprofloxacin-resistant MRSA lineages, which are mostly healthcare-associated. Although limited by a small and heterogenous patient population, this study suggests an effect of pathogen-associated factors on the success rates of MRSA eradication treatments as well. These pathogen-associated factors potentially interact with host factors, and this complex host-pathogen interaction adds to the likelihood of successfully eradicating individual MRSA carriers, in addition to the effectivity of the antimicrobial eradication treatment. A more individualized treatment approach could potentially be achieved with a deeper understanding of the genetic determinations and host-pathogen interaction.

In conclusion, there is room for improvement in every step of the cascade of MRSA care, in order to optimize the continuity of the cascade (Figure 1). These different targets at different levels underscore the importance of taking a comprehensive view when addressing potential healthcare improvements, and applying it to the local situation.

Figure 1: The cascade of MRSA care with potential targets for improvement



Legend. ID =infectious diseases, HCW = healthcare workers

Challenges in *Staphylococcus aureus* bacteremia management

The management of patients with *S. aureus* bacteremia is a complex challenge for healthcare professionals. Once the pathogen has entered the bloodstream, *S. aureus* has the potential to cause devastating damage to the human body. Uncomplicated *S. aureus* bacteremia does exist, but is very difficult to distinguish from an early phase of complicated disease and probably less prevalent than previously thought [7]. Many uncertainties need to be addressed to make decisions in diagnostic- and treatment paths. While managing uncertainties is inherent to practicing medicine, the erratic course of *S. aureus* bacteremia can amplify the usual burden of unpredictability. Even with the best available treatments, complications such as kidney injury or persistent bacteremia frequently occur in these patients. The mortality risk is high and has not substantially decreased in the past decades [8]. For equally lethal diseases such as coronary artery disease, mortality has significantly decreased following largely standardized management by guidelines, based on data from randomized controlled trials [9, 10]. Unfortunately, no such thing has happened yet for *S. aureus* bacteremia.

In **chapter 6**, we conducted a survey on the management of *S. aureus* bacteremia. This study illustrated the strength of using social media and being part of a professional network to understand global medical practices: within 20 days, over 2,000 physicians from 71 countries responded to the survey. In terms of content, the study showed that even the most basic aspects of treating patients with this disease differ profoundly between geographic regions. Differences existed in first-choice antibiotics for methicillin-susceptible *S. aureus* (MSSA) bacteremia, addition of rifampin for prosthetic device infections, the use of a 18F-FDG PET/CT scan, and route of antibiotic administration. Moreover, the definition of 'persistent SAB' varied widely between continents, ranging from two days to over seven days of positive blood cultures.

The lack of a global standard in the management of *S. aureus* bacteremia could be a result of the limited clinical trials with robust data. Despite its frequent occurrence, fewer than 3500 patients have been enrolled in published *S. aureus* bacteremia randomized trials over the past 20 years [11]. Apart from scarcity of clinical evidence, other factors such as cultural differences, type of healthcare insurance, (out-of-pocket) costs, and availability of resources also potentially influence the heterogeneity of management. Multinational clinical trials such as the *Staphylococcus aureus* Network Adaptive Platform (SNAP) are thus essential to standardize clinical definitions, identify treatment strategies, and improve patient outcomes of this common and frequently lethal infection [11]. Identifying a broadly accepted definition of persistent *S. aureus* bacteremia would not only be helpful in clinical decision-making, but also in harmonizing the terminology and outcomes used in clinical research.

In **chapter 7**, we focused on acute kidney injury in patients with *S. aureus* bacteremia. The main finding of the study was the high overall incidence of acute kidney injury. Furthermore, we observed an early development of kidney injury with a median time to peak creatinine of three days after first positive blood culture. Reversibility occurred in the majority of patients and was mostly seen in the first seven days. The early onset and swift recovery of renal insufficiency suggest that hemodynamic deterioration early in the disease plays an important role, and makes toxicity of antibiotic therapy as the primary cause of renal failure less likely. Insight in the pathogenesis of acute kidney injury in *S. aureus* bacteremia has important diagnostic and therapeutic consequences. Currently, kidney injury is often incorrectly ascribed to beta-lactam-induced tubulointerstitial nephritis, triggering an antimicrobial switch to a less potent agent. Prospective studies that focus on the different causal mechanisms of acute kidney injury in patients with *S. aureus* bacteremia are warranted to minimize unnecessary deviation from optimal therapy. Urine biomarkers potentially have additional value herein, and are a current subject of research. Different biomarker profiles may reflect prerenal or structural renal damage, and subsequently guide the clinician in the decision to change antibiotic treatment or focus on hemodynamic optimalization.

As a result of the low MRSA carriage prevalence in the Netherlands, MRSA bacteremia is exceptional in our country. However, in endemic regions such as the United States, MRSA bacteremia is common. Consequently, persistence of MRSA bacteremia despite appropriate antimicrobial treatment is also more frequently encountered. **Chapter 8** reviewed the literature on persistent MRSA bacteremia, addressing relevant host and pathogen factors. Clinical risk factors in persistent MRSA bacteremia include the retention of implanted devices and presence of metastatic infection. Potential host genetic variation and biomarkers indicative of MRSA bacteremia have recently been identified and show promise for future diagnostic options. Key genetic and phenotypic characteristics of *S. aureus* that have been associated with persistent SAB are accessory gene regulator dysfunction, variation in virulence factor production and phenotypes, antibiotic tolerance and reduced vancomycin susceptibility [12-14]. Considering treatment, vancomycin was the only recommended therapy for MRSA bacteremia for decades. Due to unfavorable safety profiles, many combinations of antibiotics have not been able to replace vancomycin. Since 2011, daptomycin is included in the guideline for MRSA bacteremia in the United States, but not in Europe. Although high-quality data is lacking, high-dose daptomycin (with a second antibiotic agent to prevent treatment-induced resistance), and the addition of ceftaroline, are currently regarded as 'best practice treatment' in persistent MRSA bacteremia [15]. Future therapeutical options may include ceftobiprole, dalbavancin, or non-antibiotic therapies such as bacteriophages.

Challenges in the management of *S. aureus* bacteremia can also arise in the form of identifying which patients are more at risk for dying than others. Ideally, in such a heterogenous disease, risk factors for mortality are known for every individual patient, guiding treatment plans and communication with patients and their relatives. Previously identified risk factors for mortality in patients with *S. aureus* bacteremia include increasing age, infective endocarditis, hemodialysis dependence and persistent bacteremia [16]. On top of these, female sex has been suggested as risk factor for mortality in several studies, even with reports of an increased mortality risk of 30% in females relative to males [17-19]. However, other studies did not find any sex-related mortality difference [20, 21]. Hence, the true influence of female sex on mortality remains unknown. Perhaps, the historical tendency to include fewer female patients in scientific studies has contributed to this knowledge gap. In **chapter 9**, we analyzed sex-differences in a large prospective cohort of *S. aureus* bacteremia patients in the United States. We found no difference in mortality between females and males. However, other characteristics differed significantly. For example, females were more often black, hemodialysis dependent, more likely to have implanted foreign material, and more likely to have used corticosteroids in the past month compared to males. Females were also more often infected with MRSA (as opposed to MSSA), compared to males. Although the aforementioned differences between females and males are interesting, they are pre-existing upon entry and therefore not potential targets for improvement.
This in contrast to differences in disease management, which were also notably present. Transesophageal echocardiography was performed less often in females. Furthermore, females were treated with a shorter median duration of antibiotics compared to male patients. The interpretation of these differences in disease management is complex, since males were also shown to have higher rates of metastatic infections, and different directions of causality are therefore plausible. More invasive diagnostic tests (i.e., transesophageal echocardiography) in males could have led to more frequent identification of complicated disease, and subsequently longer courses of antibiotics. Conversely, males could have truly had more complicated disease and therefore more often a true indication for transesophageal echocardiography. A sex-driven bias in management is therefore not downright proven in our study, but the findings warrant additional research to identify the underlying mechanisms of these discovered differences.

Given the contradictory reports in literature with regard to female sex as risk factor for mortality, we assessed all studies reporting mortality in *S. aureus* bacteremia stratified by sex in **chapter 10**. In this systematic review and meta-analysis, 89 studies with a total of 132,582 patients with *S. aureus* bacteremia were included. An increased odds of death of 18% in females relative to males was identified in this study. This difference remained when only studies that adjusted mortality for

patient and disease characteristics were included. Although almost entirely based on observational studies with a different primarily aim than assessing sex-differences, and with a risk of publication bias (inherent to meta-analyses), the sex-difference in mortality found in this study calls for further investigation.

Underlying causes of the higher mortality in females with *S. aureus* bacteremia were not addressed in our study, but it is tempting to speculate on the variety of potentially contributing factors. A biological survival disadvantage in females with *S. aureus* bacteremia is not immediately apparent, as males have generally worse outcomes in sepsis. However, female mice were more susceptible to lethal toxic shock caused by *S. aureus* enterotoxin B than male mice [22]. On a social level, a delay in health-seeking has been described in women with myocardial infarction [23], and could be present in *S. aureus* bacteremia as well. Differences in response to treatment may play a role, since both pharmacokinetics and pharmacodynamics are generally subject to sex influences [24]. Most disturbing would be a gender bias in healthcare delivery, which has been reported for example in women with septic shock, who experienced delays in antibiotic treatment relative to men [25]. Taking the results from chapter 9 in consideration, a gender bias in healthcare delivery is not yet excluded as a potential explanation for the sex difference in mortality in patients with *S. aureus* bacteremia.

Concluding remarks

Decolonization of MRSA carriership can be optimized on the levels of identification of carriers, treatment initiation, and treatment efficacy. Treatment goal and likelihood of successful prolonged eradication – driven by individual risk factors for treatment failure and risk of recolonization in the environment – should guide the eligibility for MRSA decolonization treatment in the individual patient. Future research would gain clinical applicability from reporting the carrier status of household contacts, long-term follow-up cultures, and reporting genotyping in case of failure. In order to maintain a low MRSA prevalence, the potential leakages of the MRSA cascade of care should be addressed. The details of this cascade may vary between countries, but the impact of MRSA extends beyond borders.

Large practice variations for *S. aureus* bacteremia exist throughout the world, emphasizing the complex challenge of managing this heterogeneous disease. Complications such as acute kidney injury and persistent bacteremia frequently occur in patients with *S. aureus* bacteremia, and their management is for a large part based on clinical experience rather than robust data. Female sex is a risk factor for mortality in *S. aureus* bacteremia, and the underlying cause should be unraveled. In a disease as common and frequently lethal as *Staphylococcus aureus* bacteremia, it is essential to internationally standardize clinical definitions and identify treatment strategies in order to improve patient outcomes.

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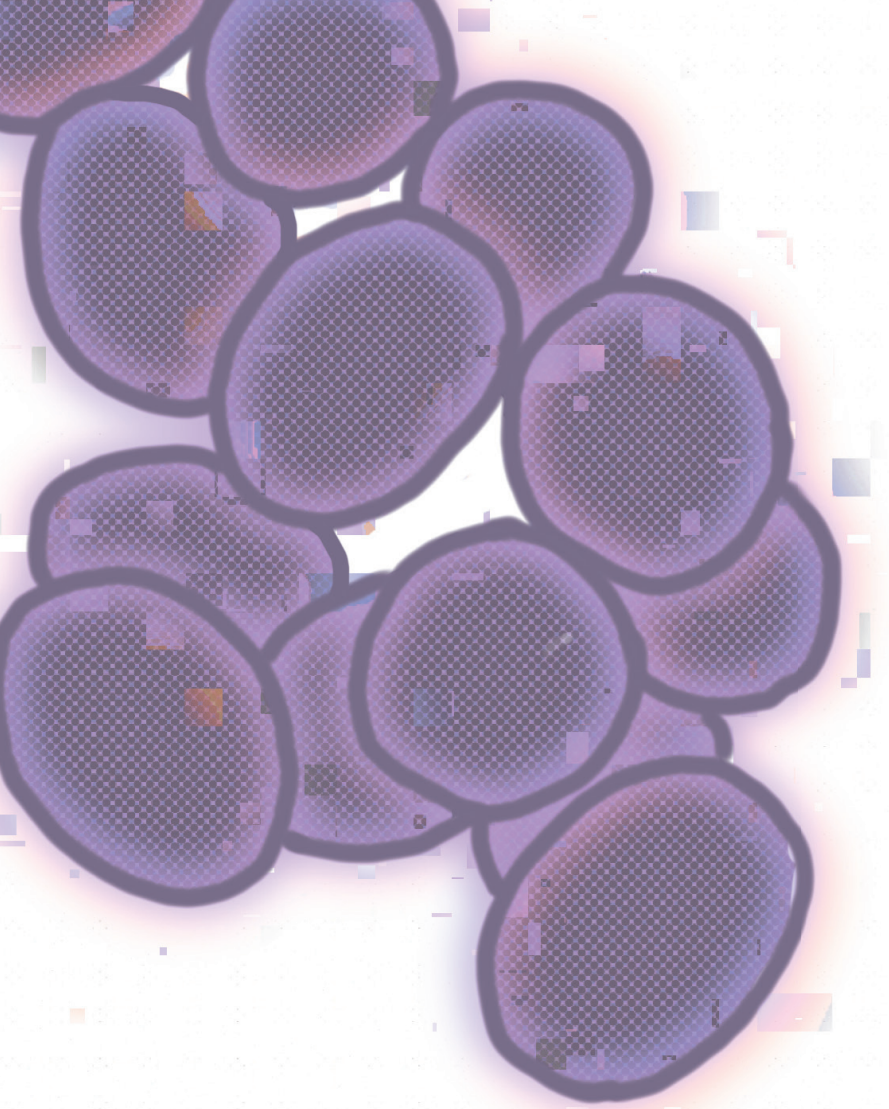

Nederlandse samenvatting

Staphylococcus aureus is een fascinerende en veelzijdige bacterie, die zijn naam dankt aan de goudkleurige koloniën op de bloedplaat en de gelijkenis met druiventrosjes onder de microscoop. *S. aureus* koloniseert een groot deel van de gezonde populatie in onder meer neus, keel, perineum, huid en darmen, bij de meeste dragers zonder symptomen te veroorzaken. Als de barrières van huid of slijmvliezen echter zijn beschadigd, kan de bacterie een heel scala aan ziektes veroorzaken. Dat kunnen veelvoorkomende en relatief onschuldige huidinfecties zijn, zoals impetigo (krentenbaard) en folliculitis (haarwortel-ontsteking). Aan de andere kant van het spectrum veroorzaakt *S. aureus* zeer ernstige infecties, zoals endocarditis (infectie van de hartklep), spondylodiscitis (infectie van de tussenwervelschijf) en bacteriëmie (bloedbaaninfectie), die leiden tot catastrofale ziekte en niet zelden tot de dood. Een belangrijke complicerende factor bij de behandeling van infecties met *S. aureus* is de potentie om antimicrobiële resistentie te ontwikkelen. Meticilline-resistente *Staphylococcus aureus* (MRSA) werd voor het eerst beschreven in de jaren ’60, kort na de introductie van het antibioticum meticilline. Moderne moleculaire technieken geven echter aanwijzingen dat MRSA door natuurlijke selectie al voorkwam in het pre-antibiotische tijdperk, en dat het wijdverspreide gebruik van penicilline en later meticilline alleen zorgden voor de juiste omstandigheden waaronder de bacterie zich kon verspreiden. MRSA was in 2019 wereldwijd verantwoordelijk voor meer dan 100,000 doden, en is daarmee uitgegroeid tot de nummer één doodsoorzaak die is toe te schrijven aan antimicrobiële resistentie. Het wordt daarom gezien als een serieuze bedreiging van de publieke gezondheid.

Ondanks de hoge prevalentie en de wereldwijde ziektelast van *S. aureus* zijn er nog veel onbeantwoorde vragen rondom de behandeling en de risicofactoren van zowel dragerschap als bloedbaaninfecties. Dit proefschrift richt zich in het eerste deel op het optimaliseren van MRSA-dragerschapsbehandeling, en in het tweede deel op veelvoorkomende uitdagingen in de behandeling van *S. aureus*-bacteriëmie.

Het optimaliseren van MRSA-dekolonisatie

Dragerschap van MRSA veroorzaakt vaak geen klachten, maar verhoogt wel de kans op het ontwikkelen van een infectie. Behandeling van MRSA-dragerschap (eradicatie- of dekolonisatie-behandeling) is bewezen effectief in het voorkómen van infecties. We mogen trots zijn op de MRSA-cijfers in ons land, die tot de laagste ter wereld behoren. Toch verdient MRSA ook in Nederland onze continue aandacht, gezien de toename van MRSA in onze buurlanden, de immigratie van mensen uit endemische gebieden en het heen en weer reizen van mensen naar deze gebieden. Het feit dat

MRSA weinig voorkomt in ons land is voor een groot deel te danken aan ons 'search and destroy'-beleid. Dit beleid houdt in dat we risicopatiënten screenen en isoleren, en MRSA-dragerschap behandelen. Het doel van dit beleid is MRSA-kolonisatie te minimaliseren, om zo verspreiding en infecties te voorkómen.

Het succes van dit beleid hangt af van verschillende opeenvolgende stappen. De eerste stappen zijn de identificatie van MRSA-dragers en het starten van een eradicatiebehandeling. In hoofdstuk 2 beschrijven we ons onderzoek naar de bekendheid van huisartsen met het 'search and destroy'-beleid, en barrières voor de toepassing ervan. Opvallend weinig huisartsen bleken bekend met het beleid rondom MRSA in Nederland, of met de MRSA-eradicatierichtlijn. Daarnaast werden er verscheidene redenen gegeven om af te zien van dragerschapsbehandeling. De meest voor de hand liggende verbeteringen in deze stappen zijn daarom het vergroten van de bekendheid met het beleid, en het opnemen ervan in de huisartsenrichtlijn.

Het is belangrijk te benadrukken dat bovenstaande studie zich specifiek richt op de Nederlandse situatie, en niet direct te vertalen is naar andere landen. De grote variatie in het voorkomen van MRSA over de wereld heeft een aanzienlijke invloed op de rationale achter dragerschapsbehandelingen, zoals we beschrijven in hoofdstuk 3. Door een hoog risico op rekolonisatie (herbesmetting) is er in een situatie met veel MRSA een lagere kans om langdurig MRSA-vrij te blijven na een dragerschapsbehandeling. In die setting leidt het 'search and destroy'-beleid zoals wij dat kennen waarschijnlijk niet tot een vermindering van het aantal dragers. In landen waar MRSA endemisch is, is het daarom passender om de focus te leggen op kortdurende verlaging van de bacteriële load, om de kans op ziekenhuisbesmettingen en postoperatieve infecties te verkleinen. Naast de kans op rekolonisatie, bemoeilijken individuele risicofactoren voor het falen van een eradicatiebehandeling de kans op een geslaagde eradicatie op lange termijn. Zowel het behandeldoel als de kans op blijvende eradicatie zouden daarom moeten worden meegenomen in de beslissing om wel of geen eradicatiebehandeling te starten in een individuele patiënt.

Als laatste 'stap' in het 'search and destroy' beleid draagt de effectiviteit van de eradicatiebehandeling bij aan het succes van het beleid als geheel. Ook in hoofdstuk 3 beschrijven we de effectiviteit van de verschillende behandelstrategieën. De combinatie van mupirocine neuszalf en desinfecterende zeep is zeer effectief in het dekoloniseren van neusdragerschap, maar lijkt onvoldoende werkzaam in patiënten die gekoloniseerd zijn op andere plekken dan de neus. Het meeste bewijs in deze patiëntencategorie is er voor een combinatie van topicale therapie (antimicrobiële neuszalf en desinfecterende zeep) met rifampicine en een tweede systemisch werkend antibioticum. De toepasbaarheid in de praktijk van de studies over MRSA-dragerschap wordt echter negatief beïnvloed door het frequente ontbreken van gegevens over dragerschapsstatus van huisgenoten, lange termijn vervolgkweken,

en genetische diagnostiek bij nieuwe positieve MRSA-kweken na behandeling. Dit laatste kan helpen onderscheid te maken tussen het falen van de behandeling of rekolonisatie met een andere bacteriestam.

Om inzicht te krijgen in de MRSA eradicatiebehandelingen in onze regio, hebben we de effectiviteit van de behandeling in vijf naburige ziekenhuizen geëvalueerd in de studie die hoofdstuk 4 vormt. We vonden een hoog succespercentage van dragerschapsbehandelingen, met het hoogste aantal successen in de groep patiënten die behandeld was met rifampicine en doxycycline. Het betrof echter retrospectief onderzoek in een relatief kleine groep, dus het voordeel van deze combinatie is hiermee niet direct bewezen. Om dit te verhelderen voeren we momenteel de CLEANEST studie uit, een prospectief onderzoek in 11 ziekenhuizen naar de meest effectieve systemische behandeling van MRSA-dragerschap.

Ook verschillen in de MRSA-bacterie zelf kunnen wellicht bijdragen aan de kans op succesvolle dragerschapsbehandeling. In hoofdstuk 5 beschrijven we onze exploratieve studie naar genetische karakteristieken van MRSA-stammen en hun effect op de succeskans van de behandeling. We vonden een hogere kans op falen in patiënten die gekoloniseerd waren met een ziekenhuis-gerelateerde stam. Alhoewel het een kleine heterogene groep betrof, suggereert deze studie wel dat verschillen aan de kant van het pathogeen ook van invloed zijn. Beter begrip over de impact van deze genetische verschillen in de verwekker zou kunnen bijdragen aan een meer geïndividualiseerde behandeling van MRSA-dragers in toekomst.

Concluderend is er ruimte voor verbetering in alle verschillende stappen van MRSA dragerschapsbehandeling, en benadrukt dit het belang van een holistische benadering bij de aanpak van verbeteringen in de gezondheidszorg en de lokale toepassing hiervan.

Uitdagingen in het management van *Staphylococcus aureus* bacteriëmie

Wanneer *S. aureus* (zowel de meticilline-resistente MRSA als de niet-resistente MSSA) eenmaal de bloedbaan is binnengedrongen, kan de bacterie tot desastreuze ziekte leiden met uitgebreide infectiehaarden door het hele lichaam. Ongecompliceerde *S. aureus*-bacteriëmie bestaat ook, maar is moeilijk te onderscheiden van een vroege fase van gecompliceerde ziekte en waarschijnlijk zeldzamer dan gedacht. Het hele spectrum bij elkaar zorgt voor een incidentie van ongeveer 30 per 100,000 persoonsjaren, en heeft een mortaliteit van 20-30%. Er wordt al decennialang onderzoek gedaan naar deze ziekte, waardoor we bijvoorbeeld weten dat een consult van een infectioloog, herhaalde bloedkweken en het routinematig maken van hartecho's allemaal bijdragen aan betere uitkomsten voor de patiënt. Toch zijn er

nog vele onzekerheden rondom de behandeling van *S. aureus*-bacteriëmie en komen complicaties zoals acute nierinsufficiëntie en persisterende bacteriëmie ondanks adequate behandeling veel voor. De heterogeniteit en onvoorspelbaarheid van deze ziekte maken het een complexe uitdaging voor zorgprofessionals en vormen een barrière voor consensus over de beste behandeling.

Dat er geen wereldwijde standaard is voor de behandeling blijkt wel uit hoofdstuk 6. Voor dat onderzoek hebben we via sociale media een wereldwijde enquête uitgezet onder zorgprofessionals over de medische praktijk rondom *S. aureus* bacteriëmie. De kracht van sociale media en een professioneel netwerk werd hiermee mooi geïllustreerd: binnen 20 dagen hadden ruim 2000 artsen uit 71 landen gereageerd. De studie liet zien dat er grote verschillen zijn tussen regio's op het gebied van eerste keus antibiotica, de toevoeging van rifampicine in het geval van geïnfecteerd kunstmateriaal, het gebruik van de PET/CT scan, en de toedieningswijze van antibiotica. Daarnaast varieerde de definitie van 'persisterende bacteriëmie' enorm, van twee tot meer dan zeven opeenvolgende dagen met positieve bloedkweken. Het ontbreken van een wereldwijde standaard kan waarschijnlijk deels verklaard worden door culturele verschillen, verschillen in welvaart en in de organisatie van zorg en verzekeringen. Deels zal het echter ook een gevolg zijn van het gebrek aan hard bewijs door robuuste data. Om behandeling te standaardiseren en uitkomsten te verbeteren zijn daarom grote internationale onderzoeken nodig.

In de studie van hoofdstuk 7 onderzochten we het optreden van acute nierinsufficiëntie in patiënten met *S. aureus*-bacteriëmie. We vonden een hoge incidentie van acute nierinsufficiëntie, die meestal al heel vroeg in de ziekte optrad. In de meerderheid van de patiënten herstelde de nierfunctie weer, meestal binnen de eerste week. Het vroege optreden en snelle herstel suggereren dat hemodynamische veranderingen in het begin van de ziekte een belangrijke rol spelen, en maken toxiciteit van de behandeling als oorzaak minder waarschijnlijk. Inzicht in het ontstaan van een acute nierinsufficiëntie in *S. aureus*-bacteriëmie is belangrijk, omdat nu de oorzaak vaak onterecht wordt gezocht in de antibiotica, die vervolgens wordt vervangen door een minder effectief middel. Wellicht zijn urine-biomarkers ('signaalstoffen'), waarvan de rol nu onderzocht wordt, van toegevoegde waarde hierin. Deze biomarkers zouden kunnen helpen om onderscheid te maken tussen nierfalen door schade aan de nier zelf en een buiten de nier gelegen oorzaak.

Als gevolg van de lage prevalentie van MRSA-dragerschap in Nederland, zien wij maar zelden een bacteriëmie veroorzaakt door MRSA. Dat is anders in landen waar MRSA endemisch is, zoals de Verenigde Staten, waar MRSA-bacteriëmie aan de orde van de dag is. In die landen wordt dus ook vaker persisterende MRSA-bacteriëmie gezien, waarbij ondanks adequate behandeling de bloedkweken positief blijven. In hoofdstuk 8 hebben we de literatuur rondom persisterende MRSA-bacteriëmie

bestudeerd en samengevat, waarbij zowel gastheer- als pathogeen-geassocieerde factoren geadresseerd werden. Voor de behandeling van persisterende MRSA-bacteriëmie is vancomycine jarenlang de enige aangeraden therapie geweest. In de Verenigde Staten is in 2011 daptomycine opgenomen in de richtlijn. Vooral op basis van 'expert opinion' wordt dit nu, als ook het toevoegen van ceftaroline, aangeraden bij persisterende MRSA-bacteriëmie.

Een andere uitdaging in de behandeling van *S. aureus*-bacteriëmie ligt in de vraag welke patiënten meer risico hebben op overlijden dan anderen. Bekende risicofactoren zijn bijvoorbeeld ouderdom, endocarditis, dialyse-afhankelijkheid en persisterende bacteriëmie. Daarnaast is in sommige studies gesuggereerd dat vrouwen een grotere kans op overlijden hebben dan mannen. In hoofdstuk 9 beschrijven we ons onderzoek naar man-vrouw verschillen in een groot prospectief cohort van *S. aureus*-bacteriëmie patiënten in de Verenigde Staten. We vonden hier geen verschil in mortaliteit, maar wel veel andere verschillen, zoals dat vrouwen – vergeleken met mannen – vaker zwart waren, vaker dialyse-afhankelijk, vaker kunstmateriaal in situ hadden en vaker steroïden hadden gebruikt in de voorafgaande maand. Daarnaast waren vrouwen vaker geïnfecteerd met MRSA (in plaats van MSSA). Alhoewel dit interessante verschillen zijn, zijn deze allemaal al aanwezig bij presentatie, en daarom niet direct verbeterpunten. Dit is anders voor de verschillen in diagnostiek of behandeling, die we ook vonden. Hartecho's werden minder vaak gemaakt bij vrouwen, en vrouwen werden gemiddeld korter met antibiotica behandeld. De interpretatie van deze verschillen is echter complex, omdat mannen ook vaker gemetastaseerde infecties hadden. Meer diagnostiek in mannen zou kunnen hebben geleid tot meer diagnoses van gemetastaseerde ziekte en daarom langere behandelduur. Andersom is ook mogelijk dat mannen daadwerkelijk meer gemetastaseerde ziekte hadden, en daarom terecht vaker hartecho's en langere behandelingen hebben ondergaan. Een verschil in benadering op basis van geslacht is hiermee dus niet bewezen, maar deze bevindingen rechtvaardigen zeker extra onderzoek naar de onderliggende oorzaak van de verschillen.

Vanwege de contrasterende bevindingen over het vrouwelijk geslacht als risicofactor voor overlijden bij patiënten met *S. aureus*-bacteriëmie, hebben we een systematisch literatuuronderzoek en meta-analyse verricht (hoofdstuk 10). Hierin werden 89 studies met bij elkaar 132,582 patiënten geïncludeerd. We vonden een verhoogde mortaliteit onder vrouwen, met een verhoogde 'odds' van 18% ten opzichte van mannen. Dit verschil bleef bestaan als we alleen studies die corrigeerden voor patiënt- en ziektefactoren includeerden. Dit onderzoek is volledig gebaseerd op observationele studies die voor het grootste deel een ander doel hadden dan naar man-vrouw verschillen te kijken, maar het grote verschil in mortaliteit vraagt om nader onderzoek.

We hebben in dit onderzoek niet naar onderliggende oorzaken van het man-vrouw verschil gekeken, maar er zijn een aantal hypotheses te bedenken. Een biologisch nadeel in vrouwen is niet direct voor de hand liggend, omdat juist mannen over het algemeen slechtere uitkomsten hebben in geval van sepsis. In een muismodel bleken vrouwelijke muizen wel vatbaarder voor het ontwikkelen van een dodelijke shock door een specifiek toxine van *S. aureus* dan mannelijke muizen. Het verschil zou ook op het sociale vlak kunnen liggen, bijvoorbeeld door vertraging in het zoeken van hulp door vrouwen, wat ook is beschreven bij vrouwen met een hartinfarct. Daarnaast kan de reactie op behandeling tussen mannen en vrouwen verschillen door andere farmacokinetiek en -dynamiek. Het meest verontrustende zou een verschil zijn in de kwaliteit van geleverde gezondheidszorg aan vrouwen en mannen, zoals beschreven is in vrouwen met septische shock, die later antibiotica ontvingen dan mannen.

Conclusie

Dekolonisatie van MRSA-dragerschap kan geoptimaliseerd worden op de vlakken van identificatie van dragers, starten van dragerschapsbehandeling en effectiviteit van de behandeling. De beslissing om te starten met dragerschapsbehandeling in een individuele patiënt wordt beïnvloed door zowel het doel van de behandeling als de kans op succesvolle langdurige eradicatie. Dit laatste is afhankelijk van individuele risicofactoren op falen van de behandeling en het risico op rekolonisatie vanuit de omgeving. Toekomstig onderzoek zou baat hebben bij het vermelden van de dragerschapsstatus van huisgenoten, lange termijn controlekweken en van de genetische karakteristieken van de MRSA-stam in het geval van een nieuwe positieve kweek na behandeling.

De wereldwijde diversiteit in de benadering van *S. aureus*-bacteriëmie benadrukt de complexiteit van de behandeling van deze heterogene ziekte. Complicaties zoals acute nierinsufficiëntie en persisterende bacteriëmie komen frequent voor en hun behandeling is grotendeels gebaseerd op klinische ervaring in plaats van op robuuste data. Het vrouwelijk geslacht is een risicofactor voor mortaliteit onder patiënten met *S. aureus*-bacteriëmie, waarvan de onderliggende oorzaak uitgezocht moet worden. Het is voor een ziekte met zo een hoge prevalentie en mortaliteit als *S. aureus*-bacteriëmie essentieel om klinische definities en behandelstrategieën internationaal te standaardiseren, om uiteindelijk de uitkomsten voor patiënten te verbeteren.

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Curriculum Vitae

Annette werd geboren op 7 december 1990. In 2008 haalde ze haar gymnasiumdiploma aan de RSG Hoekschewaard in Oud-Beijerland. In datzelfde jaar startte zij met de opleiding geneeskunde aan de Universiteit Leiden. Tijdens haar studie deed ze ervaring op in het buitenland tijdens een keuzestage in Indonesië, een coschap in Thailand, en een wetenschapsstage in Spanje. In 2015 behaalde ze de master geneeskunde. Haar eerste baan was als ANIOS interne geneeskunde in het Alrijne Ziekenhuis. Daarna volgde een klein jaar als ANIOS interne geneeskunde in het Sint Elisabeth Hospitaal op Curaçao. In 2018 startte zij met de opleiding tot internist in het LUMC, met als opleiders prof. dr. De Fijter en prof. dr. Appelman. Een belangrijk deel van de opleiding volgde zij in het HagaZiekenhuis, waar haar enthousiasme voor infectieziekten en wetenschappelijk onderzoek werd aangewakkerd. In 2020 begon zij daar aan het eerste onderzoek dat uiteindelijk tot dit proefschrift leidde. Haar promotietraject heeft zij grotendeels op de afdeling infectieziekten van het LUMC uitgevoerd, onder leiding van prof. dr. De Boer, prof. dr. Visser, dr. Lambregts en dr. Schippers. In 2022 heeft zij een half jaar aan Duke University in North Carolina, Verenigde Staten gewerkt als *research scholar* op het gebied van *Staphylococcus aureus* bacteriëmie, onder leiding van prof. dr. Fowler. In 2024 begon zij aan de differentiatie infectieziekten in het LUMC. Naast haar werkzaamheden als AIOS en dit promotietraject, is zij projectcoördinator van het MRSA Netwerk Holland West en voorzitter van de Vereniging Arts-Assistenten van het LUMC.