TRANSMISSION DYNAMICS AND RESISTANCE IN STAPHYLOCOCCI

DAVID JELGER HETEM

ISBN: 978-94-6182-595-7

Layout and printing: Off Page, www.offpage.nl

Printing of this thesis was financially supported by the Department of Medical Microbiology, UMC Utrecht and the Netherlands Society of Medical Microbiology (NVMM) and the Royal Netherlands Society for Microbiology (KNVM).

Copyright 2015 by David Jelger Hetem

Transmission dynamics and resistance in staphylococci

Resistentie en verspreiding van staphylococcen
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 3 september 2015 des middags te 4.15 uur

door

David Jelger Hetem

geboren op 16 maart 1984
te Den Haag

Promotor: Prof. dr. M.J.M. Bonten

TABLE OF CONTENTS

Part I	**General introduction**	
Chapter 1	*Staphylococcus aureus* and coagulase-negative staphylococci	9
Part II	**Nosocomial transmission of methicillin-resistant *Staphylococcus aureus***	
Chapter 2	Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*	47
Chapter 3	Transmission of Methicillin-Resistant *Staphylococcus aureus* after Short-term Health Care Setting Exposure	61
Chapter 4	Nosocomial transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Danish Hospitals	69
Chapter 5	Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Thirteen European Intensive Care Units	83
Part III	**Dynamics of mupirocin resistance in *Staphylococcus aureus* and coagulase negative staphylococci**	
Chapter 6	Clinical relevance of mupirocin resistance in *Staphylococcus aureus*	103
Chapter 7	Emergence of high-level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use	119
Chapter 8	Acquisition of high-level mupirocin resistance in CoNS following nasal decolonization with mupirocin	129
Chapter 9	Prevention of surgical site infections: decontamination based on pre-operative screening for *Staphylococcus aureus* carriers or universal decontamination?	139
Part IV	**Management of intravascular catheters colonized with *Staphylococcus aureus***	
Chapter 10	Preventing *Staphylococcus aureus* bacteremia and sepsis in patients with *Staphylococcus aureus* colonization of intravascular catheters: A retrospective multicenter study and meta-analysis	161
Chapter 11	General discussion	173

Samenvatting in het Nederlands 185
Dankwoord 191
Curriculum Vitae 193
List of publications 195

part I

GENERAL INTRODUCTION

one

STAPHYLOCOCCUS AUREUS AND COAGULASE-NEGATIVE STAPHYLOCOCCI

David J. Hetem, Suzan H.M. Rooijakkers, Miquel B. Ekkelenkamp

Adapted from: Infectious Diseases, 4th edition, Edited by Cohen, Powderly and Opal.
Chapter 176: Staphylococci and micrococci

In press

NATURE

Staphylococci are Gram-positive spherical bacteria about 1 micrometer in diameter, which divide in three dimensions and, due to incomplete cell separation, form a 'bunch of grapes' cluster that defines the genus.[1] The genus *Staphylococcus* has been classified together with genera including *Bacillus*, *Gemella*, *Listeria* and *Planococcus*, in the order of Bacillales and the family of ***Staphylococcaceae***. Approximately fifty species have been described thus far (http://www.bacterio.cict.fr), which are able to colonize or infect multiple animal species. For instance: *S. hyicus* is the main causative agent of infectious dermatitis and arthritis in swine, *S. aureus* causes bovine mastitis, and has also been reported in pigs, pigeons, cats and dogs, *S. intermedius* causes infections in dogs, foxes, mink, pigeons and horses.

EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS*

Staphylococci, in particular *Staphylococcus aureus*, are ubiquitous and frequent causes of infection in humans, and have been so throughout history. As the cause of post influenza necrotizing pneumonias, *S. aureus* was considered responsible for at least a quarter of the deaths during the Spanish influenza pandemic of 1917–1918, and it is estimated that half of the casualties in the trenches of the First World War were due to septic wound infections with *S. aureus*.

In healthy humans, carriage (or colonization) of *S. aureus* may occur on multiple sites of the skin and mucosal surfaces (including the intestine and vagina), the main reservoir being the anterior nares (vestibulum nasi/ nostrils). In the general population 20% are persistently colonized, 30% intermittently colonized and the remaining 50% are non-susceptible to colonization, but colonization rates can differ extensively among healthy subjects.[2] Person-to-person spread is believed to occur mainly by direct hand/skin contact; nosocomial spread is primarily mediated by health-care workers. Furthermore, up to 10% of healthy *S. aureus* carriers disperse the bacterium into the air. Under normal circumstances, when airborne dispersers are at rest, they are surrounded by 0.01–0.1 colony-forming units/m^3 but up to 0.3 cfu/m^3 in selected cases. However, the bacterial density may increase 40-fold with movement (due to release of bacteria from the clothing) and with respiratory tract infections. Multiple outbreaks have been attributed to single airborne spreaders. Although (methicillin-resistant) *S. aureus* has been reported to persist on inanimate surfaces for up to 7 months, the role of environmental contamination or airborne transmission is controversial. Although acquisition occurs primarily on the skin, *S. aureus* can only persist in the long term if the nares or perineum become colonized. Generally, the established flora of the nose prevents the acquisition of new strains.

Patients with type 1 diabetes, patients undergoing hemodialysis, surgical patients, intravenous drug users and HIV-infected patients have an increased risk of *S. aureus* colonization. Heavy antibiotic pressure may lower (detectable) colonization rates.[2]

Methicillin-susceptible *S. aureus* (MSSA) strains have a high genetic diversity across Europe and the USA.[3,4] In contrast, methicillin-resistant *S. aureus* (MRSA) shows a high degree of geographic clustering. Based upon multilocus sequence typing (MLST) the population structure of hospital-acquired MRSA (HA-MRSA) is characterized by five major clonal complexes (CCs) with pandemic clones: CC5, CC8, CC22, CC30 and CC45 (see Table 1). Within these five clonal complexes different SCC*mec* types are found, indicating that MRSA clones emerged by multiple independent introductions of the *mecA* gene.[5]

Table 1. Most common MRSA lineages and their geographic distribution

Clonal complex	Sequence type (ST)	SCC*mec* type	Epidemic clones	Geographic distribution
HA-MRSA				
CC5	ST5	II	New York/Japan, USA100	North America, Asia, Australia, Europe
	ST5	I	UK-EMRSA-3	Europe, South America
	ST5	IV	Paediatric clone/USA800	USA, South America, Europe
	ST228	I	Southern German	Europe
CC8	ST8	IV	UK-EMRSA-2/6, USA500	North America, Europe, Australia
	ST247	I	Iberian, UK-EMRSA-5	USA, Europe
	ST239	III	Brazilian, Hungarian	Asia, Australia, South Africa, South America, Europe
	ST250	I	Archaic clone	
CC22	ST22	IV	UK-EMRSA-15	Europe, Australia, Canada
CC30	ST36	IV	UK-EMRSA-16, USA200	North America, UK, Australia
CC45	ST45	IV	Berlin, USA600	USA, Europe
CA-MRSA				
CC1	ST1	IV	USA400, WA-MRSA-1	USA, Australia
CC8	ST8	IV	USA300	North America, South America, Europe
CC30	ST30	IV	Southwest Pacific	USA, Asia, Australia, South America, Europe
CC59	ST59	VII	Taiwan	Taiwan, China
CC80	ST80	IV	European	Europe, North Africa
CC88	ST129	IV	WA-MRSA-2	Australia
CC152	ST152	V	Balkan	Europe
LA-MRSA				
CC9	ST9	V		Asia
CC398	ST398	V		Europe

Note: CC: clonal complex, ST: sequence type, MRSA: methicillin-resistant *Staphylococcus aureus,* SCC*mec:* staphylococcal cassette chromosome *mec*.

Nosocomial *Staphylococcus aureus* infections

Staphylococcus aureus is the major cause of severe nosocomial infections, and empiric treatment of such infections will always need to include *S. aureus* coverage. Nosocomial transmission of resistant *S. aureus* strains, in particular MRSA and strains with reduced susceptibility against vancomycin, convey a risk to hospitalized patients: MRSA-transmission in hospitals has been demonstrated to lead to higher rates of invasive infection with *S. aureus*.[6]

Some countries, such as The Netherlands and the Scandinavian countries, have succeeded in containing the nosocomial spread of MRSA by adhering to extensive infection-control measures and restrictive antibiotic policies, but in general MRSA-colonization is widespread in hospitals. In the USA, for instance, the proportion of MRSA infections among patients with nosocomial *S. aureus* bacteremia increased from 2.4% in 1975 to 29% in 1991, and in American intensive care units the proportion of MRSA infections had risen to nearly 60% by 2003.[7] Interestingly, in recent years a decline in the percentage of nosocomial *S. aureus* infections being MRSA has been observed in countries as the UK and the USA.[8]

Nasal carriage of *S. aureus* is a risk factor for subsequent infection in hospitalized patients. Colonized surgical patients had an absolute risk of wound infection of roughly 5–15%, which was two to eight times the risk of control patients.[9] Persistent urinary tract colonization with *S. aureus* carries a high risk for subsequent *S. aureus* infection and bacteremia. Intestinal carriage of *S. aureus* is associated with an increased risk of subsequent *S. aureus* infection when compared to nasal carriage alone.[10,11] A large observational study showed that nosocomial *S. aureus* bacteraemia was three times more frequent in *S. aureus* carriers than in non-carriers and ~80% of the strains causing *S. aureus* bacteraemia in carriers were from endogenous origin (i.e. they were colonized at admission).[12] However, mortality in case of *S. aureus* bacteraemia was higher in patients who were not colonized at hospital admission.

Community-acquired MRSA

For several decades, MRSA-colonization and -infection was largely confined to healthcare settings, but in the USA during the 1990's MRSA strains emerged which were able to sustain themselves and spread in the community. The major community-associated MRSA (CA-MRSA) clone is the USA300 strain (this number is based on pulsed-field gel electrophoresis analysis), a Panton Valentine leucocidin (PVL) positive *S. aureus*, of sequence type (ST) 8 by MLST. USA300 has a propensity to cause skin and soft tissue infections, and was at first found mostly in outbreaks, but currently this clone causes the majority of *S. aureus* infections in both hospitals and the USA community.[13] Several other CA-MRSA clones have emerged around the world, such as the European clone CC80 (*pvl*-positive, ST80) and the South-West Pacific clone (*pvl*-positive, ST30) (Figure 1).[14]

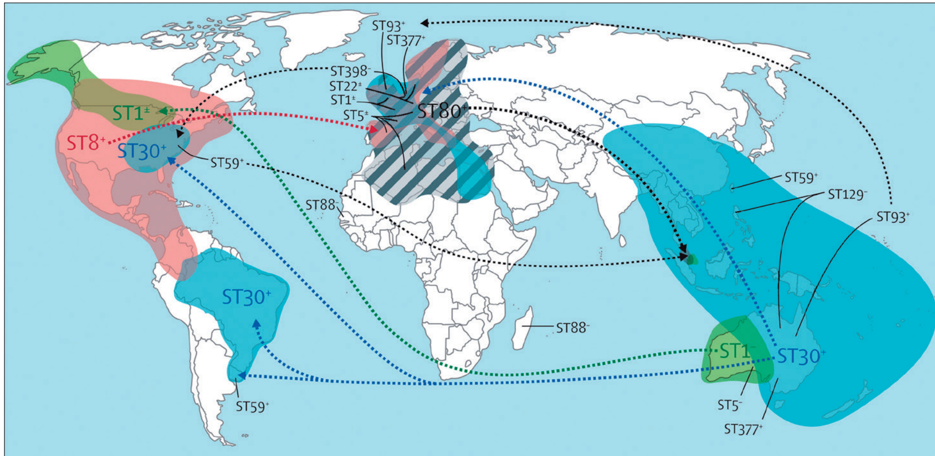


Figure 1. Global distribution of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) by multi-locus sequence type (MLST). Dotted lines indicate possible route of dissemination of the CA-MRSA strains. Estimates of the areas are shown in which infections with the main strains—i.e., ST1 (green), ST8 (red), ST30 (blue), and ST80 (grey hatched)—have been reported. + = Panton-Valentine leukocidin (PVL)-positive strains. − =PVL-negative strains. ± = combination of PVL-positive and PVL-negative strains.
Figure redrawn from: DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant Staphylococcus aureus. *Lancet* 2010, 375, 1557–1568.

Although CA-MRSA is still uncommon as a cause of disease in Europe, in several European countries MRSA is widely spread among livestock, with subsequent transmission of MRSA to caretakers. Nosocomial transmission of livestock-associated MRSA (LA-MRSA) is low, however, and hospital outbreaks are therefore rare.[15] Phylogenetically, LA-MRSA is not related to any of the major MRSA CCs: in Europe most strains belong to CC398 (ST398), whereas in South-East Asia CC9 is more common as LA-MRSA.

COAGULASE-NEGATIVE STAPHYLOCOCCI: EPIDEMIOLOGY AND INFECTIONS

Shortly after birth colonization with coagulase-negative staphylococci (CoNS) occurs, the normal habitats of these staphylococci being the skin and the mucous membranes. *S. epidermidis* is the predominant species; other frequent colonizers include *S. hominis*, *S. haemolyticus* and *S. warneri*. Although in general CoNS are nonpathogenic colonizers, their propensity to adhere to biomaterials and form biofilms makes them important causative agents of foreign body-related infections. CoNS are the main pathogens isolated in catheter-associated bloodstream infections and drain-associated meningitis. They are

amongst the foremost causes of prosthetic joint infections and prosthetic heart valve endocarditis, and they cause ~10% of all cases of native valve endocarditis.[16] *S. epidermidis* is primarily responsible for foreign body infections, and both prosthetic and native valve endocarditis. *S. saprophyticus* causes urinary tract infections and *S. lugdunensis* and *S. schleiferi* may cause infections very similar to those of *S. aureus*, including abscesses, endocarditis and wound infections. Infections in humans with *S. intermedius* are rare and have been associated with exposure to animals, in particular dogs.

The colonizing CoNS flora may be influenced by antibiotic therapy. In hematology and neonatology wards with high antibiotic pressure, a population of more resistant and/or more virulent CoNS strains may be selected and become epidemic among patients and health-care workers: multiple reports describe outbreaks of *S. epidermidis* clones causing intravascular catheter-related bloodstream infections.[17]

PATHOGENICITY OF *STAPHYLOCOCCUS AUREUS*

The broad spectrum of diseases caused by *S. aureus* is due to its production of many surface-bound and extracellular virulence factors. These include molecules that adhere to host tissues, counteract the host defense or lyse cells (Table 2).

Adherence to host cells and tissues

Staphylococcus aureus expresses a number of adhesion molecules that facilitate interactions with host cells and extracellular matrix (ECM) components. These 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs) are surface-anchored molecules that bind host molecules like collagen, laminin, fibronectin, elastin, vitronectin and fibrinogen.[18] MSCRAMMS are involved in colonization and sepsis, and also mediate the attachment of *S. aureus* and *S. epidermidis* to foreign body materials and indwelling devices because coating of the biomaterials with host proteins and platelets results in biofilm formation (Figure 2).[19]

Blocking host defenses

The immune response against *S. aureus* largely depends on the innate immune system: antimicrobial peptides, the complement system and phagocytes. The bacterium, in response, produces highly specific proteins that enable it to suppress the immune response.

Resistance to antimicrobial peptides

In response to infectious stimuli, skin keratinocytes, mucosal epithelial cells and neutrophils produce high levels of antimicrobial peptides (AMPs) known as cathelicidins (LL-37) and defensins. The *S. aureus* metalloproteinase aureolysin cleaves LL-37, while staphylokinase (SAK) inhibits the bactericidal effect of α-defensins.[20] Furthermore, modification of cell wall teichoic acids promotes *S. aureus* resistance to AMPs.[21]

Table 2. Virulence factors of *Staphylococcus aureus*

Immune evasion mechanisms		
Virulence factor	**Acronym or gene**	**Activity**
Clumping factor	ClfA, ClfB	Binds fibrinogen, coating the bacterial cell and inhibiting phagocytosis
Chemotaxis inhibitory protein of *S. aureus*	CHIPS	Downregulates the C5a receptor and the formylated peptide receptor (FPR) on neutrophils; inhibits chemotaxis
Extracellular adherence protein	EAP	Binds to ICAM-1, fibrinogen, vitronectin Blocks leukocyte adhesion, diapedesis and extravasation
Extracellular fibrinogen-binding protein/extracellular complement binding protein	Efb/Ecb	Bind to C3 molecules, inhibit convertases. Efb creates an anti-phagocytic fibrinogen shield
Staphylococcal complement inhibitor	SCIN/ SCIN-B/ SCIN-C	Inhibit C3 convertases, inhibiting C3b deposition and phagocytosis
Staphylokinase	SAK	Activates human plasminogen at the bacterial surface to cleave opsonins; inhibits bactericidal effect of α-defensins
Staphyloxantin (golden pigment)		Resist oxidant killing
Polysaccharide capsule		Antiphagocytic function
FLPR1 inhibitory proteins	FLIPr/ FLIPr-like	Block Fc receptors, impair neutrophil responses to formylated peptide receptor-like-1 agonists
Polysaccharide intercellular adhesin	PIA	Holds multilayered cell complexes that form biofilms together, decreases susceptibility to defensins
Catalase		Inhibits bacterial killing by inactivating hydrogen peroxidase and free radicals formed by the myeloperoxidase system within phagocytic cells
Protein A	SpA	Binds Fc part of human IgG and prevents phagocytic uptake by Fc receptors; stimulates B lymphocytes
Coagulase/von Willebrand binding protein	*coa/ vwbp*	Bind and activate prothrombin into thrombin
Staphylococcal superantigen-like 3	SSL3	Inhibits TLR2 activation
Staphylococcal superantigen-like 5	SSL5	Binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling
Staphylococcal superantigen-like 7	SSL7	Binds IgA and blocks FcαRI-mediated responses. Binds C5 and blocks C5 cleavage into C5a and C5b
Staphylococcal superantigen-like 10	SSL10	Binds IgG and inhibits FcR recognition and complement activation

Table 2. Virulence factors of *Staphylococcus aureus (Continued)*

Immune evasion mechanisms		
Virulence factor	**Acronym or gene**	**Activity**
Aureolysin	Aur	Metalloproteinase that cleaves LL-37 and complement C3
Staphylococcal immunoglobulin-binding protein	SBI	Binds IgG and C3. Blocks complement activity
Staphopain A	ScpA	Cleaves CXCR2 and blocks chemotaxis
Invasion mechanisms		
α-Hemolysin	*hla*	Lyses macrophages, lymphocytes and erythrocytes
β-Hemolysin	*hlb*	Sphingomyelinase; damages eukaryotic cell membranes containing sphingomyelin; causes lysis of sheep erythrocytes on blood agar
γ-Hemolysin	*hlgA, hlgB*	Consists of two proteins which assemble to form membrane-perforating complexes; toxic to PMNs, monocytes and macrophages, lytic for red blood cells
Panton–Valentine leukocidin	*lukS (lukS-PV, lukF-PV)*	Lysis human neutrophils after binding to the C5a receptor
Leukocidin E/D	*LukED*	Lytic to leukocytes
δ-Hemolysin	*hld*	Variety of attributed actions: multimerizes on eukaryotic membranes to form lytic pores; possible mediator of staphylococcal membranous enterocolitis; linked to atopic dermatitis by activating mast cells
Exfoliative toxins	*eta, etb*	Epidermolytic proteases that cleave desmoglein. Cause of staphylococcal scalded skin syndrome
Fibrinolysins		Break down fibrin clots
Hyaluronidase	*hysA*	Hydrolyzes intercellular matrix of mucopolysaccharides
DNAse/thermonuclease	*nuc*	Hydrolyzes RNA and DNA, frees nutrients
Lipase	*geh*	Facilitates spread in subcutaneous tissues; associated with furunculosis
Superantigens/pyrogenic exotoxins		Stimulate T cells nonspecifically to cytokine release
Enterotoxins A, B, C, D, E, G, H, K (and others)	*sea, seb, sec, sed, see, seg, seh, sek*	Cause staphylococcal food poisoning and half of the cases of nonmenstrual toxic shock syndrome (TSS)
Toxic shock syndrome toxin	TSST-1/*tst*	Responsible for about 75% of cases of TSS, including all cases of menstrual TSS

Table 2. Virulence factors of *Staphylococcus aureus (Continued)*

Microbial surface components recognizing adhesive matrix molecules		
MSCRAMM	**Gene**	**Activity**
Fibronectin-binding protein	*fnbpA, fnbpB*	Binds fibronectin, fibrinogen and elastin
Collagen-binding protein	*cna*	Binds collagen/cartilage
Clumping factor	*clfA, clfB*	Binds fibrinogen

ICAM-1, intercellular adhesion molecule 1; PMNs, polymorphonuclear leukocytes; PSGL-1, P-selectin glycoprotein ligand-1.

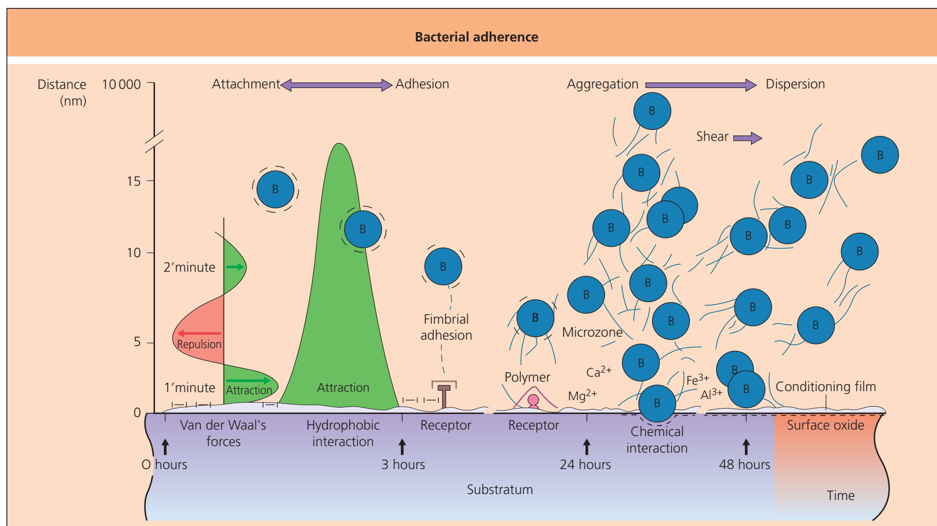


Figure 2. Biofilm formation. The figure illustrates the events associated with bacterial (B) adherence to a biomaterial in relation to time and the molecular sequence in bacterial attachment, adhesion, aggregation and dispersion at substratum surface. A number of possible interactions may occur depending on the specificities of the bacteria or substratum system, the distance from the biomaterial and the stage of adherence. The attachment stage is mediated by nonspecific forces. Adhesion is driven by specific adhesin–receptor interactions. The final aggregative step results in a bacterial macrocolony on the biomaterial surface in which the bacteria are firmly adherent to the biomaterial and each other. Bacterial exopolysaccharide blankets the macrocolony and may serve to improve the nutritional microenvironment and protect the bacteria from host defenses. In the dispersion phase, bacteria disaggregate, break loose from the macrocolony and drift free into the bloodstream. Adapted with permission from Gristina AG. Biomaterial centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588.© 1987 American Association for the Advancement of Science.

Complement evasion

The complement cascade serves three major functions in innate immunity:

- to opsonize bacteria (through C3b) (Figure 3);
- to attract phagocytes (through C3a and C5a); and
- to perturb bacterial membranes of Gram-negative bacteria (C5b–9, the membrane attack complex or MAC).[22,23]

Complement activation is initiated by three different pathways (classical, lectin or alternative) that all result in the formation of C3 convertase enzymes that cleave the central complement protein C3 (Figure 4). The C3 cleavage product C3b covalently binds to the bacterial surface and is recognized by phagocytic cells expressing complement receptors. Furthermore, C3b associates with C3 convertases to form a C5 convertase that cleaves C5 into C5a (a potent chemoattractant) and C5b (part of the MAC). *S. aureus* produces a variety of molecules that interfere with multiple steps of the complement cascade (Table 2). For instance, the secreted staphylococcal complement inhibitor (SCIN) blocks C3 convertases to interfere with C3b deposition and phagocytosis,[24] while staphylococcal superantigen-like (SSL) protein 7 specifically binds to C5 to prevent cleavage by C5 convertases and formation of C5a.[25]

Mutagenesis studies have indicated that staphylococcal complement inhibitors contribute to the pathogenesis of *S. aureus* in vivo.[26]

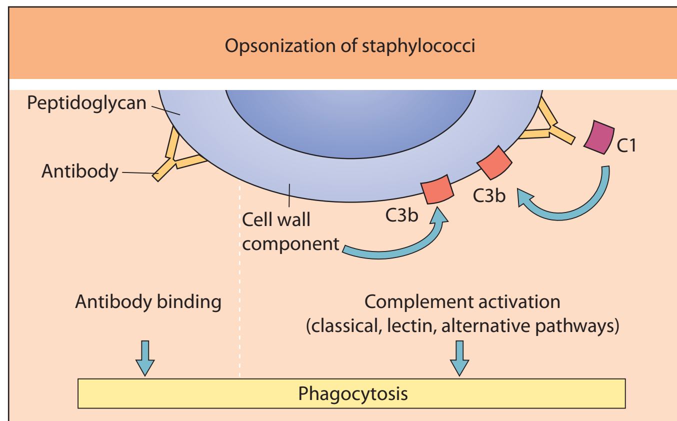


Figure 3. Opsonization of staphylococci with antibody molecules and complement activation products is essential for effective phagocytosis. Antibodies bind to the surface and are recognized by Fc receptors on phagocytic cells. Complement activation, either triggered by antibodies or cell wall components, results in labeling of bacteria with C3b and iC3b, which are recognized by complement receptors.

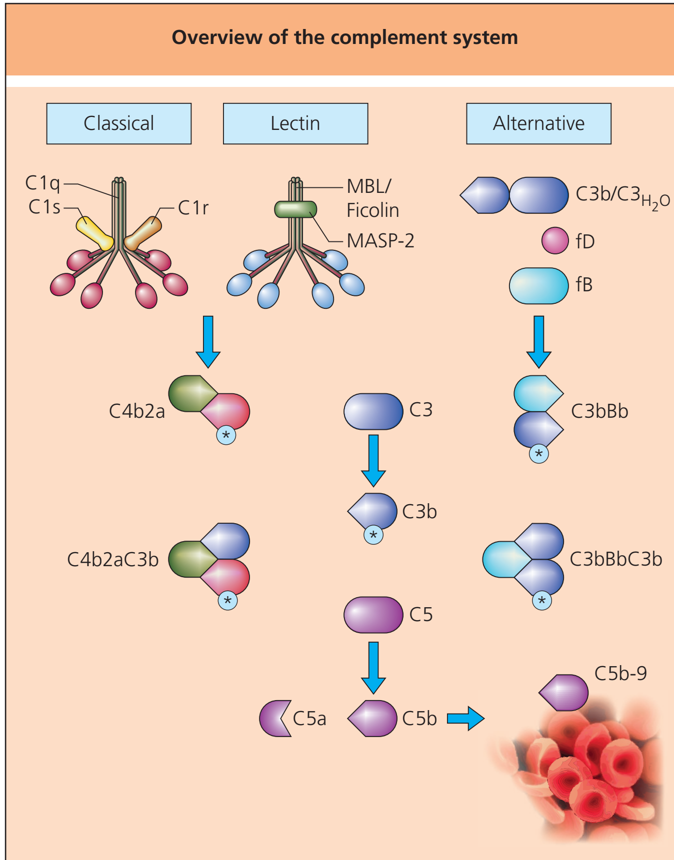


Figure 4. Schematic overview of the complement system. Complement activation can occur via three different pathways. The antibody-dependent classical pathway starts when C1q in the C1q–$C1r_2$–$C1s_2$ complex recognizes antibodies that are bound to the microbial surface. In the lectin pathway, mannose binding lectin (MBL) and ficolins recognize microbial sugar patterns and activate the MBL-associated serine protease 2 (MASP-2). Both C1s and MASP-2 can cleave complement proteins C4 and C2 to generate the CP/LP C3 convertase, C4b2a. Within this complex, C4b is covalently (*) attached to the microbial surface. The alternative pathway C3 convertase (C3bBb) is generated after binding of factor B (fB) to surface-bound C3b or fluid-phase C3(H_2O). Factor B is subsequently cleaved by factor D (fD) to generate C3bBb. Both C3 convertases C4b2a and C3bBb cleave C3 into covalently bound C3b (*) and an anaphylatoxin C3a. C3b contributes to phagocytosis, antigen presentation and formation of C5 convertases, C4b2a3b and C3bBb3b. C5 convertases cleave C5 into an anaphylatoxin C5a and C5b, which forms a complex with complement proteins C6, C7, C8 and C9 to generate the membrane attack complex (MAC) and mediate microbial lysis.

Inhibition of neutrophil recruitment and activation

Effective eradication of *S. aureus* depends on phagocytosis and intracellular killing by immune cells, mainly neutrophils.[27] This critical role is reflected by the increased risk for *S. aureus* infections in patients with defects in granulocyte function, both inherited (e.g. chronic granulomatous disease, myeloperoxidase deficiency, leukocyte adhesion deficiencies) and acquired (e.g. diabetes mellitus, rheumatoid arthritis, HIV). During an infection, neutrophils are rapidly recruited from the circulation to sites of microbial invasion by host stimuli (complement fragment C5a, interleukin 8, leukotriene B4) and pathogen-derived stimuli (fMLP, phenol-soluble modulins (PSMs)).[28] These chemotactic factors activate neutrophils, increase vascular permeability and induce expression of adhesion molecules on endothelial cells. Neutrophils express selectins and integrins that bind these adhesion molecules; the cells start to roll on the endothelial lining and firmly adhere to it.[29] Subsequently, the neutrophils migrate through the endothelial cell layer (diapedesis) and move towards the site of infection under a gradient of chemoattractant substances.

S. aureus secretes several molecules that specifically block phagocyte recruitment: the staphylococcal superantigen-like 5 (SSL5) inhibits neutrophil rolling by blocking the interaction between P-selectin on endothelial cells and P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils;[30] the chemotaxis inhibitory protein of *S. aureus* (CHIPS) prevents chemotaxis by blocking the formylated peptide receptor and the C5a receptor20 and the cysteine protease Staphopain A cleaves the chemokine receptor CXCR2.[31] *S. aureus* also prevents neutrophil activation by the staphylococcal superantigen-like protein 3 that binds Toll-like receptor 2.[32]

Resistance to phagocytosis and intracellular killing

S. aureus is most efficiently phagocytosed after opsonization by both complement and antibodies. Upon bacterial uptake, the interaction of opsonic ligands with receptors triggers the release of oxygen radicals and granular contents (e.g. myeloperoxide, proteases) into the phagosome that can destroy the ingested particle.[33] *S. aureus* resists phagocytosis by expression of complement inhibitory proteins that decrease surface deposition of C3b (Table 2). Furthermore, the Extracellular Fibrinogen binding protein (Efb) effectively inhibits phagocytosis by covering bacteria in an anti-phagocytic shield of fibrinogen (Figure 6).[34] Some *S. aureus* strains surround themselves with a loose-fitting polysaccharide capsule (Figure 5) that hinders the binding of surface-bound complement factors to phagocyte receptors.[35] Alternatively, *S. aureus* specifically modulates Fc-dependent uptake by secretion of the Formyl Peptide Receptor-like 1 Inhibitor (FLIPr) protein family that potently bind and antagonize Fc receptors on neutrophils. The *S. aureus* surface protein A (SpA) also blocks antibody dependent phagocytosis by since this protein binds the Fc terminal of human IgG and covers the bacterial surface with outward-facing IgG molecules that cannot react with Fc receptors (Figure 5).

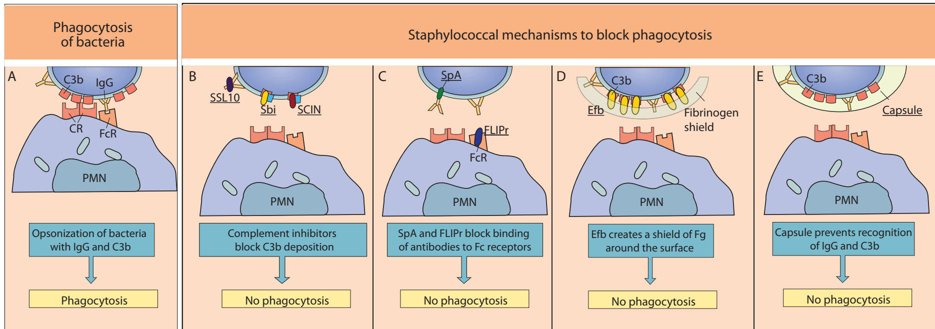


Figure 5. Staphylococcal mechanisms to escape phagocytosis. (A) Phagocytosis requires opsonization of bacteria with antibodies (IgG) and complement fragments (C3b) (B-E) *S. aureus* uses different mechanisms to block phagocytosis: (B) Complement inhibitors block the labeling of *S. aureus* with C3b; (C) surface-bound SpA binds Fc domains of IgG, thereby orienting the molecule in the wrong orientation and FLIPr/FLIPr-like bind the Fc receptor (D) Efb binds C3b and attracts Fibrinogen that hides opsonins from phagocyte receptors; (E) The capsule prevents recognition of opsonins on the cell wall of *Staphylococcus aureus* to complement and Fc receptors on PMNs.

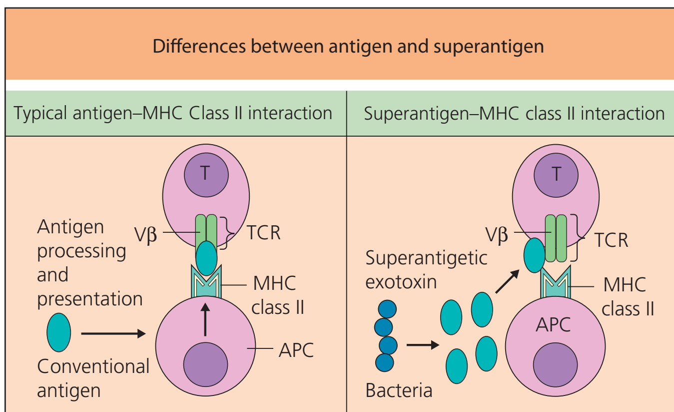


Figure 6. Differences between antigen and superantigen. Staphylococcal enterotoxin and TSST-1 act as superantigens, binding directly to MHC class II and the Vb chains of the T-cell receptor (TCR) without the need for normal antigen processing. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

Once phagocytosed, staphylococci may inhibit killing and travel through the bloodstream within neutrophils. The golden pigment staphyloxanthin (for which *S. aureus* is named) is a carotenoid molecule with antioxidant properties that scavenges free oxygen radicals.[36] Furthermore, *S. aureus* can resist oxidative stress by two superoxide dismutase enzymes that remove superoxide.

Cytolytic toxins and proteases

S. aureus secretes a variety of cytotoxins that lyse host cells by forming β-barrel pores in cytoplasmic membranes. These toxins are secreted as monomers but form multimeric pores in the membrane of target cells (Table 2). The five different bicomponent pore-forming leukocidins all 'recognize' their target cells via G-protein coupled receptors. The best-known leukocidin, Panton–Valentine leukocidin (PVL), specifically binds the C5a receptors on human neutrophils to cause lysis before the bacteria are engulfed.[37] PVL is well-known for to its association with furunculosis and hemorrhagic pneumonia and is strongly associated with recent outbreaks by community-associated MRSA strains (CA-MRSA).[27] *S. aureus* can also lyse neutrophils after engulfment via PSMs.[38] Delta toxin, also part of the PSM family, was recently identified as a potent mast cell degranulation factor causing allergic skin disease.[39]

Immunostimulatory molecules

Superantigens (or pyrogenic exotoxins) are the agents responsible for toxic shock syndrome (TSS) (Figure 6). These extracellular proteins bind to the exterior surface of major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs), and link them to receptors on the surface of T-helper cells, activating them without the need for antigen presentation by the APCs.[27]

Toxic shock syndrome toxin 1 (TSST-1) causes most cases of TSS, including all cases of tampon-associated TSS; approximately one-fourth of the cases are caused by enterotoxins. Apart from their superantigenic activity, when ingested orally the heat-resistant enterotoxins may also cause *S. aureus* food poisoning, characterized by emesis with or without diarrhea. The target responsible for initiating the emetic reflex is located in the abdominal viscera, where putative (unidentified) cellular receptors for the enterotoxins exist.

Interactions with the coagulation system

S. aureus produces two extracellular coagulase (coagulase and von Willebrand binding protein) which bind and activate prothrombin into thrombin. The activated thrombin converts fibrinogen to fibrin, causing localized clotting and shielding the bacteria from host defenses.[40] In addition, most strains express a fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue.

Genetic location and regulation of virulence factors

S. aureus virulence factors can be chromosomally encoded and uniformly present, or located on mobile genetic elements such as bacteriophages, plasmids, transposons and pathogenicity islands. The genes for exfoliative toxins A and B are located on a bacteriophage and a plasmid respectively (0–2% of strains), PVL is located on a bacteriophage (2% of isolates). The pathogenicity island harboring TSST-1 is found in 14–24%. The immune modulators CHIPS, SCIN, SAK and SEA are clustered bacteriophage present in 90% of clinical *S. aureus* isolates.[41]

The expression of virulence factors in *S. aureus* is controlled by a complex system of regulatory mechanisms. A well-studied response regulator is the accessory gene regulator (*agr*), a two-component quorum sensing system which switches the preferential expression of surface adhesins during the exponential growth phase to the expression of secreted proteins during the post exponential and stationary growth phases.[42] This system is turned on at high bacterial densities and recent studies showed that *agr* is turned on when bacteria are inside the phagolysosomal vacuole of the neutrophil. There, *agr* drives expression of PSMs that subsequently lyse the neutrophil.[43] Another important regulator is the SaeRS system that drives expression of most immune evasion molecules.[44]

PATHOGENICITY OF *STAPHYLOCOCCUS EPIDERMIDIS*

Most of the pathogenicity studies in coagulase-negative staphylococci (CoNS) have focused on virulence factors involved in foreign-body infections by the most common and relevant species, *Staphylococcus epidermidis*. These infections are characterized by the formation of biofilms: first the bacteria adhere to the foreign body or indwelling device, followed by an accumulation phase in which the bacteria form multilayered cell clusters embedded in extracellular material.

Hydrophobic interactions, Van der Waal's forces and bacterial surface proteins play a role in initial bacterial adherence to the foreign material. On insertion or implantation, the material is rapidly coated with plasma proteins and extracellular matrix proteins (e.g. fibronectin, fibrinogen, vitronectin, von Willebrand factor), providing additional attachment sites. Molecules which mediate attachment to polymers include the staphylococcal surface proteins SSP-1 and SSP-2, the surface-associated autolysin AtlE, biofilm-associated protein (Bap) and the capsular polysaccharide/adhesin (PS/A). Molecules which bind to extracellular matrix proteins include fibrinogen-binding protein (Fbe, a protein with similarity to ClfA in *S. aureus*), cell-wall techoic acid (attachment to fibronectin) and AtlE (binds to vitronectin). A number of factors involved in the accumulation phase have been identified: the polysaccharide intercellular adhesin (PIA), also known as slime-associated antigen (SAA); the capsular polysaccharide/adhesin (PS/A); biofilm-associated protein (Bap); and accumulation-associated protein (AAP).[45] Elastases, proteases, lipases and fatty-acid modifying enzymes have been identified in *S. epidermidis* and are considered possible virulence factors.[45]

PREVENTION

S. aureus is the main pathogen causing post-operative wound infections. Strict compliance with prophylactic antibiotic regimens for surgery (i.e. timely administration) and adaptation of these regimens to the local susceptibility patterns are essential to maximally reduce such infections.

Prevention of MRSA/spread

During the 1980s several countries implemented nationwide 'search-and-destroy policies' to limit the spread of MRSA within hospital settings. At that time carriage of MRSA among hospitalized patients was still extremely low. The cornerstone of the search-and-destroy policies is that colonized patients are treated in strict isolation; admitted patients with an increased risk of MRSA carriage are screened (see below) and isolated until culture results rule out MRSA carriage. Finally, contact patients and healthcare workers are screened for MRSA carriage in case of unexpected detection of MRSA in a hospitalized patient. In the United Kingdom multiple MRSA prevention initiatives were implemented to reduce the spread of MRSA in hospitals; the effects assessed via a mandatory MRSA bacteraemia reporting scheme. Mandatory reporting of MRSA bacteraemia showed a reduction in MRSA infections of more than 50% between 2003 and 2010.[46]

Prevention of hospital-acquired infections by decolonization

Peri-operative eradication of *S. aureus* carriage, using mupirocin nasal ointments and chlorhexidine body washings for 5 days, may reduce the number of nosocomial *S. aureus* infections by up to 60%.[47] Topical mupirocin is highly effective for short-term nasal eradication of *S. aureus*; 90% of patients remain negative after 1 week, and around 60% after a longer follow-up period.[48]

In smaller populations, such as CAPD patients, hemodialysis patients and patients with recurrent skin infections, mupirocin treatment was associated with significant reductions in *S. aureus* infections.[49]

DIAGNOSTIC MICROBIOLOGY

The name 'staphylococcus' (dervived from the Greek σταφυλή, a bunch of grapes) was introduced by Alexander Ogston, a Scottish surgeon who in 1881 described the presence of grape-like clusters of spherical micro-organisms in pus from abscesses.[50] The first to isolate and culture staphylococci was the German surgeon Friedrich Rosenbach. Rosenbach distinguished two different species of staphylococci based on colony color: a species with yellow/orange/golden colonies which he named *Staphylococcus aureus* (derived from the word aurum, gold in Latin), and a species with white colonies which he called *Staphylococcus albus* that was later renamed *Staphylococcus epidermidis*.

Staphylococci are nonmotile, nonspore-forming bacteria with a genome size of between 2000 and 3000 kbp, and a 30–39% GC-content. Most staphylococcal species demonstrate catalase activity and are facultative anaerobes. Only *S. aureus* subspp. *anaerobius* and *S. saccharolyticus* require anaerobic conditions for growth. Further characteristics of the genus include susceptibility to furazolidone, resistance to bacitracin, and production of acid from glucose under anaerobic conditions or in the presence of erythromycin.

The main constituents of the staphylococcal cell wall are peptidoglycan, which constitutes 50% of the dry cell mass, and teichoic acid (40% of the dry cell mass). The glycan chains of the peptidoglycan layer are built with approximately 10 alternating subunits of *N*-acetylmuramic acid and *N*-acetylglycosamine. Pentapeptide side chains are attached to the *N*-acetylmuramic acid subunits; the glycan chains are then cross-linked with peptide bridges between the side chains. The teichoic acids are macromolecules of phosphate containing polysaccharides. Teichoic acid is bound both to the peptidoglycan layer (Wall Teichoic acids, WTA) and to the cytoplasmic membrane (Lipoteichoic acid, LTA). The polysaccharides are species specific; *S. aureus* cell walls contain ribitol teichoic acids while *S. epidermidis* makes glycerol teichoic acids.

Isolation and determination

Most staphylococcal lesions contain numerous polymorphonuclear leukocytes (PMNLs) and large numbers of *S. aureus*, which may readily be demonstrated by a direct Gram smear of pus (Figure 7). Direct Gram smears of sputum samples may also assist in rapid identification of staphylococcal pneumonia.

In general, staphylococci grow overnight on most conventional bacteriologic media. The preferential medium for isolation is (sheep) blood agar, on which they form colonies of 2 mm or more in diameter (Figure 8). Blood cultures from untreated bacteremia patients are usually positive after overnight incubation. Staphylococci may grow at a temperature range of 15–45°C and at NaCl concentrations as high as 15%. Differentiation from other Gram-positive cocci may be aided by the determination of a couple of characteristics (Table 3). The fermentation of mannitol by *S. aureus* is used in mannitol salt agar to screen for this bacterium in clinical and environmental samples.[51]

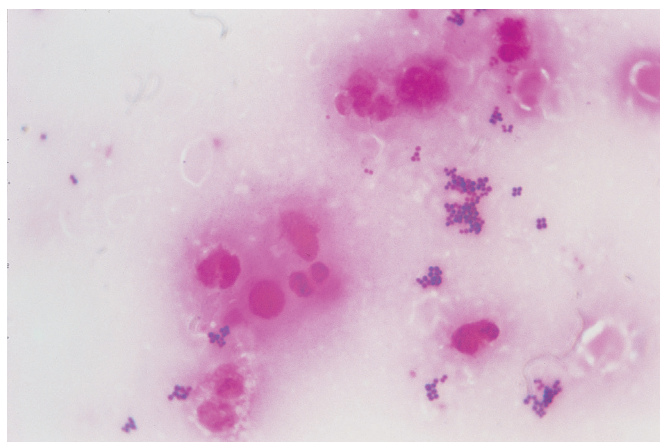


Figure 7. *Staphylococcus aureus* in a Gram stain of pus. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

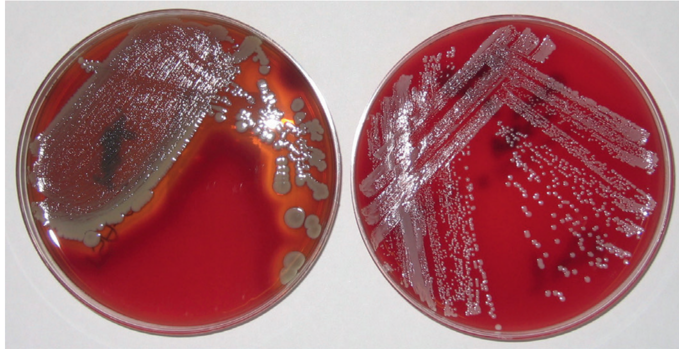


Figure 8. Growth of *Staphylococcus aureus* (left) and *Staphylococcus epidermidis* (right) on trypticase soy agar with sheep blood.

Table 3. Differentiation of *Staphylococcus aureus* and coagulase negative staphylococci from other Gram-positive cocci

	Staphylococcus aureus	CoNS	*Micrococcus* spp.	*Kocuria kristinae*	*Rothia mucilaginosa*
Gram stain	Gram-positive cocci, in clusters	Gram-positive cocci, in clusters	Gram-positive cocci, in clusters	Gram-positive cocci in tetrads	Gram-positive cocci in pairs or clusters with capsules
Color	Cream colored to golden	White to cream	Cream colored to canary yellow	Cream colored to canary yellow	Clear to white
Mupirocin	Susceptible	Susceptible or resistant	Resistant	Resistant	–
Bacitracin	Resistant	Resistant	Susceptible	Susceptible	Susceptible
Growth in 6.5% NaCl	Yes	Yes	Yes	Yes	No
Oxidase	Negative	Negative	Positive	Positive	Negative
Catalase	Positive	Positive	Positive	Positive	Weakly positive or negative
Coagulase	Positive	Negative	Negative	Negative	Negative

Note: CoNS: coagulase negative staphylococci.

S. aureus colonies on blood agar can be differentiated from other staphylococci by their yellowish (gold-colored) pigment. Confirmation tests include latex agglutination assays that detect protein A and clumping factor ('bound coagulase') on the cell surface of *S. aureus* (Figure 9), testing for free coagulase and for DNAse/thermostable endonuclease. However, non-optimal sensitivity of these tests has been reported, especially in identifying MRSA.

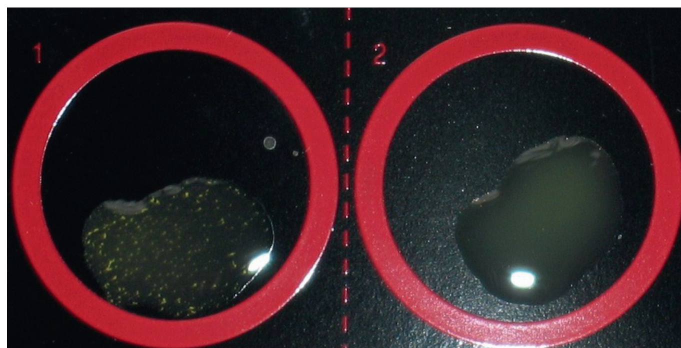


Figure 9. Slide coagulase test. Latex particles coated with fibrinogen and IgG agglutinate when a colony of *Staphylococcus aureus* is suspended in the solution (left), and negative control (right).

Most CoNS species can be determined with carbohydrate utilization tests and enzyme tests (e.g. phosphatase, urease, nitrate reduction). *S. saprophyticus* from urine samples may be identified by demonstrating novobiocin resistance.

The introduction of matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry in microbiological labs facilitated the identification and differentiation of staphylococcal species, as MALDI-TOF can rapidly and accurately identify staphylococci and discriminate between *S. aureus* and CoNS species.[52]

Phenotypic susceptibility testing

S. aureus susceptibility testing can be performed by disc diffusion or E-test on several standard bacteriologic media, and by microbroth or macrobroth dilution. Guidelines and breakpoints are available from the Clinical and Laboratory Standards Institute (CLSI), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). A number of automated systems are available for broth dilution susceptibility testing. These tests are adequate for most antibiotics, but certain special considerations apply (see below). Susceptibility profiles of different *S. aureus* strains can differ extensively (Table 4).

Clindamycin susceptibility testing

Methylation of the ribosomal target, usually encoded by *ermA* or *ermC*, is the main mechanism of resistance against clindamycin, and also results in cross-resistance to macrolides, lincosamide and streptogramin B (MLS_B) (Table 5).[53] Clindamycin does not induce expression of these methylase genes in vitro and tested strains may wrongly appear susceptible. An induction test with erythromycin (erythromycin and clindamycin disk placed 20–26 mm from each other) should, therefore, be performed on erythromycin-resistant *S. aureus* strains.

Table 4. Susceptibility profiles of different *Staphylococcus aureus* strains

	MSSA (Miko et al.)		**MRSA (Diekema et al.)**	
	Outpatient (N=298)	**Inpatient (N=410)**	**HA-MRSA USA100 (N=368)**	**CA-MRSA USA300 (N=2093)**
Antimicrobial agent				
Clindamycin	97	92	5	91
Erythromycin	67	70	2	8
Levofloxacin	90	87	3	47
Trimethoprim-sulfamethoxazole (TMP-SMZ)	100	99	98	99
Daptomycin	100	100	99	100
Linezolid	100	100	100	100
Vancomycin	100	100	100	100

Note: Data are percentage of isolates being susceptible. MSSA: methicillin susceptible *Staphylococcus aureus*, MRSA: methicillin resistant *Staphylococcus aureus*, HA-MRSA: hospital associated methicillin resistant *Staphylococcus aureus*, CA-MRSA: community associated methicillin resistant *Staphylococcus aureus*, TMP-SXT: trimethoprim-sulfamethoxazole. CLSI breakpoints were used.

Table 5. Resistance genes and resistance mechanisms for *Staphylococcus aureus*

Antibiotic	**Resistance Gene(s)**	**Gene Product(s)**	**Mechanism(s) of Resistance**	**Location(s)**
β-Lactams	*blaZ*	β-Lactamase	Enzymatic hydrolysis of β-lactam nucleus	Plasmid: Transposon
	mecA	PBP2a	Reduced affinity for PBP	Chromosome: SCC*mec*
Glycopeptides	GISA: unknown	Altered peptidoglycan	Trapping of vanco-mycin in the cell wall	Chromosome
	VRSA: *vanA*	D-Ala-D-Lac	Synthesis of dipeptide with reduced affinity for vancomycin	Plasmid: Transposon
Quinolones	*parC*	ParC (or GrlA) component of topoisomerase IV	Mutations in the QRDR region, reducing affinity of enzyme-DNA complex for quinolones	Chromosome
	gyrA or *gyrB*	GyrA or GyrB components of gyrase		
Aminoglycosides (eg, gentamycin)	Aminoglycoside-modifying enzymes (eg, *aac*, *aph*)	Acetyltransferase, phosphotransferase	Acetylating and/or phosphorylating enzymes modify aminoglycosides	Plasmid: Transposon

Table 5. Resistance genes and resistance mechanisms for *Staphylococcus aureus (Continued)*

Antibiotic	Resistance Gene(s)	Gene Product(s)	Mechanism(s) of Resistance	Location(s)
Trimethoprim-sulfamethoxazole (TMP-SMZ)	Sulfonamide: *sulA*	Dihydropteroate synthase	Overproduction of *p*-aminobenzoic acid by enzyme	Chromosome
	TMP: *dfrB*	DHFR	Reduced affinity for DHFR	
Tetracyclines	Tetracycline, doxycycline and minocycline: *tetM*	Ribosome protection protein	Binding to the ribosome and chasing the drug from its binding site	Plasmid: Transposon
	Tetracycline: *tetK*	Efflux protein	Efflux pump	Plasmid
Erythromycin	*msrA*	Efflux protein	Efflux pump	Plasmid
	erm (A, C)	Ribosomal methylase (constitutive or inducible)	Alteration of 23S rRNA	Plasmid: Transposons
Clindamycin	*erm (A, C)*	Ribosomal methylase (constitutive or inducible)	Alteration of 23S rRNA	Plasmid: Transposons
Linezolid[a]	*cfr*	Ribosomal methyltransferase	Methylation of the 23S rRNA that interferes with ribosomal binding	Plasmid
Daptomycin[b]	*mprF*	Lysylphosphatidylglycerol synthetase (LPG) synthetase	Increasing: synthesis of total LPG, outer LPG translocation and positive net charges on cell membrane	Chromosomal
Mupirocin[C]	*mupA*	Alternative isoleucyl-tRNA synthetase (*ILeRS-II*)	Reduced affinity for mupirocin	Plasmid

Table adapted from Clinical infectious diseases: Stryjewski ME, Corey GR. Methicillin resistant *S. aureus* An Evolving pathogen, 2014.

Note: DHFR, dihydrofolate reductase; GISA, glycopeptide-intermediate susceptible *Staphylococcus aureus*; LPG, lysylphosphatidylglycerol; QRDR, quinolone resistance–determining region; VRSA, vancomycin-resistant *S. aureus*. [a] Other mechanisms for linezolid resistance involve mutations to the central loop of domain V of 23S rRNA or in the ribosomal proteins L3 and/or L4 of the peptide translocation center. [b] Other mechanisms were also proposed, such as increased cell wall thickening, decreased membrane fluidity, and increased expression of *vraSR*. [c] High-level resistance is mediated by *mupA*, whereas low-level resistance is results from a point mutation in the native chromosomal *IleRS*

Isoxazolyl penicillins (oxacilline, cloxacillin, flucloxacillin, nafcillin)

Methicillin resistance results from the production of an alternative penicillin-binding protein, PBP2A (or PBP2'), encoded by the *mecA* gene on the staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element, supposedly acquired through horizontal gene transfer from CoNS (Table 5). Although the gold standard for identification of MRSA is in fact the detection of the *mecA* gene, recently a homologue to *mecA*, called *mecC,* has been detected in MRSA from human and bovine origin.[54,55] In heterogeneous MRSA populations, expression of PBP2a may be suppressed in most colony-forming units, hindering detection by disk diffusion or by automated (microbroth dilution) systems. A screening assay with 30 μg cefoxitin disks has the highest sensitivity for MRSA detection, with specificity being comparable to other susceptibility assays.[56] Screening for MRSA colonization can be performed on selective media (both liquid and solid) containing either oxacillin or cefoxitin. Several chromogenic MRSA detection media are available which contain an indicator agent to distinguish *S. aureus* from CoNS. Sensitivity and specificity of most of these tests are reported to be higher than 90–95%.[57]

In some cases, overexpression of penicillinases may lead to resistance against isoxazolyl penicillins. These strains do not harbour the *mecA*-gene and may have MICs in the susceptible range for betalactam/betalactamase-inhibitor combinations. They are not considered "true" MRSA and have thus far not been associated with outbreaks.

Glycopeptides

To reliably determine the susceptibility of staphylococci for glycopeptides MIC-testing should be performed, as disk diffusion has insufficient sensitivity and specificity. Vancomycin-intermediately susceptible *S. aureus* (VISA), defined by a minimum inhibitory concentration (MIC) of >2 and ≤8 mg/l, is associated with thickening of the bacterial cell wall, thereby creating an excess of binding sites to 'trap' vancomycin.[58,59] Vancomycin susceptible strains stably producing subcolonies (at a frequency of ≥1 / 10^6 according to the population analysis profile) with MICs in the VISA range are called heterogeneous vancomycin intermediate resistant *S. aureus* (hVISA); these are difficult to detect using standard laboratory methods. VISA isolates can return to susceptible strains in the absence of antibiotic pressure of vancomycin.[60] Both VISA and hVISA phenotypes are associated with an impaired clinical response to vancomycin.[61,62]

High-level vancomycin-resistant *S. aureus* (VRSA) is extremely rare (MIC ≥16 mg/l) and results from acquisition of the enterococcal *vanA* resistance gene by *S. aureus*. Recently, a vancomycin resistant CA-MRSA strain phylogenetically related to USA300, was reported from Brazil.[62] Worryingly, the strain carried a plasmid containing *vanA* cluster which was readily transmissible to other staphylococci. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) considers that it is unclear whether increased doses of vancomycin improve clinical outcome in infections with VISA, and therefore does not differentiate between VISA and VRSA. EUCAST recommends reporting all *S. aureus* with an MIC>2 as VRSA.

Most vancomycin resistant strains are also resistant against teicoplanin. Furthermore, resistance against vancomycin has also been associated with reduced susceptibility to daptomycin.[63]

Linezolid

Linezolid resistance in *S. aureus* and CoNS has been detected in patients previously treated with linezolid and has been reported in nosocomial outbreaks from several countries. Linezolid resistance is associated with mutations in the 23S rRNA or the presence of a transmissible *cfr* ribosomal methyltransferase.[64]

Genotypic susceptibility testing

On demand automated rapid cartridge based amplification assays can identify *S. aureus* and MRSA carriers within 3-4 hours. These systems can also identify *S. aureus* and MRSA from cultured colonies, clinical samples taken from skin and soft tissue infections, and from positive blood cultures vials containing Gram-positive cocci. Polymerase chain reaction (PCR) targets are usually the *mecA* gene in combination with a specific *S. aureus* gene (e.g. the *spa* gene, the coagulase gene (*coa*) or the nuclease gene (*nuc*)). However, none of these techniques is 100% sensitive or specific. MRSA isolates containing the *mecC* gene can result in false negative PCR results.

The main *S. aureus* genes in conferring resistance against antimicrobial agents are described in table 5. Applying such resistance-databases, considerable progress has been made with whole-genome sequencing (WGS) to predict a susceptibility profiles of bacterial strains.[65]

Typing methods

The epidemiology of (methicillin-resistant) *S. aureus* may be studied by typing the isolated strains. Numerous typing methods are available, differing in reproducibility, cost, ease, speed and discriminatory capacity.

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PGFE) is based on the digestion of bacterial DNA with restriction endonucleases (for MRSA usually *smaI*), generating large fragments of DNA (10–800 kb). PFGE has a high discriminatory power and the results are highly reproducible. However, there are limitations to its use, such as the long time interval until the final results are obtained, limited transferability, multiple nomenclatures and the cost of reagents and specialized equipment.

Multilocus sequence typing

Multilocus sequence typing (MLST) characterizes bacterial isolates by using the sequences of internal fragments of seven housekeeping genes.[66] Every polymorphism of a housekeeping gene is assigned a number, yielding a code consisting of seven numbers for each bacterial isolate; subsequently, each new code receives a sequence type (ST) number. Advantages of

MLST include its unambiguous nomenclature, easy global exchange of typing data and the possibility for population structure and evolutionary analyses. On the downside, MLST is less discriminatory than PFGE and more expensive.

Multilocus variable number tandem repeat analysis

Multiple locus variable number tandem repeat analysis (MLVA) uses the variability in the number of short tandem repeat sequences to create DNA profiles for epidemiological studies. Multiple MLVA schemes have been designed and used for *S. aureus*. MLVA has lower costs than MLST, can be as discriminatory as PFGE, and has an improved resolution compared to *spa* typing.[67] Limitations include the absence of an international protocol for MLVA and the absence of universal nomenclature.

Spa typing

Spa typing is a single-locus sequence typing technique for *S. aureus*, based on the polymorphic region X of the protein A gene.[68] *Spa* typing is highly reproducible and easy to interpret. It has less discriminatory power than PFGE and MLVA, but is less costly and easier to perform. A web-based reference database (http://www.spaserver.ridom.de), which uses a standardized spa-type nomenclature, permits global epidemiologic comparison of isolated MRSA strains.

Whole genome sequencing

Recent technological advances have made whole genome sequencing (WGS) of bacteria more accessible and affordable. The latest generation of sequencing platforms can produce a whole genome sequence of a bacterium within 24 hours. The increased resolution of WGS can disprove transmission events which were otherwise indicated by conventional methods and can also reveal otherwise unsuspected transmission events. The increased resolution is especially useful in countries and healthcare centers with a single dominant strain.[69] However, the lack of universal nomenclature still hampers the comparison between laboratories and with historical isolates. Currently, *S. aureus* core genome allele-based typing, based on a standardized analysis of whole genome sequences are being developed.[70] Thus, enabling comparisons between historical and current isolates by WGS. Next to providing insight in transmission events, WGS may provide comprehensive information about the presence of resistance genes and virulence factors.

CLINICAL MANIFESTATIONS OF STAPHYLOCOCCUS AUREUS INFECTIONS

S. aureus is an invasive micro-organism with a propensity for abscess formation. Community-acquired infections mostly involve skin and soft tissue infections such as cellulitis and furunculosis, but also pneumonia (typically post-influenza), osteomyelitis and acute endocarditis. *S. aureus* is the most common causative agent of infective endocarditis,

accounting for 28% and 21% of the native valve and prosthetic valve endocarditis cases, respectively.[16] Staphylococcal toxins may be responsible for food poisoning, staphylococcal toxic shock syndrome (TSS) and staphylococcal scalded skin syndrome (SSSS).[71]

In nosocomial settings *S. aureus* is the main causative agent of postoperative wound infections, often leading to abscess formation. It is notorious for infecting prosthetic materials, such as prosthetic joints, prosthetic heart valves and internal pacemakers. Furthermore, it is one of the main causes of intravascular catheter-associated bloodstream infections (CR-BSI), causing 10% of all CR-BSI, second only to CoNS with 34% of the CR-BSI caused.[72] *S. aureus* is also a frequent cause of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). *S. aureus* bacteremia (SAB), although more a symptom than a disease, is often regarded as a specific clinical entity due to its associated mortality risk and high rate of relapses and complications.[73,74]

Infrequently, *S. aureus* causes urinary tract infections, predominantly in patients with recent urinary tract surgery or other manipulations, and in patients with urinary tract obstruction.[10]

MANAGEMENT OF STAPHYLOCOCCUS AUREUS INFECTIONS

Management of *S. aureus* infections involves the combination of source control and antibiotic therapy. Uncomplicated wound or skin and soft tissue infections should be treated locally by drainage (after incision in case of abscess formation or necrotectomy in case of necrosis) or local antiseptics. Systemic antibiotics may be required if there is severe cellulitis or associated deep tissue infection. First choice for systemic therapy are the narrow-spectrum beta-lactams such as the isoxazolyl penicillins or first-generation cephalosporins. Alternative (oral) regimens include co-trimoxazole, clindamycin (used especially in the treatment of abscesses, for its high tissue penetration) or linezolid; these agents are usually also active against CA-MRSA. The main characteristics of antimicrobial agents effective against *S. aureus* and MRSA are described in table 6.

β-Lactam antibiotics are the agents of first choice in the treatment of (severe) systemic methicillin-sensitive *S. aureus* (MSSA) infections. Comparative studies between different β-lactam antibiotics are lacking, as are studies evaluating different durations of treatment. Isoxazolyl penicillins, penicillin/β-lactamase inhibitor combinations, first- and second-generation cephalosporins and carbapenems are considered equally effective in the treatment of MSSA infections. Clinical experience with the isoxazolyl penicillins and their narrow spectrum of activity makes them the first choice of therapy. Vancomycin, a glycopeptide, has been the antibiotic of choice for (severe) systemic infections with MRSA and in patients with β-lactam allergy. The glycopeptides are significantly less active than the β-lactams,[75] and trough levels should be monitored to ensure adequate (high enough) dosing

of vancomycin in patients with severe infections. Vancomycin-induced nephrotoxicity may occur after longer durations of administration.[76] In the majority of cases nephrotoxicity is reversible, and patients seldom require dialysis. Recent studies suggest daptomycin may be a preferred to treat patients failing on vancomycin therapy and patients whose infections are caused by strains with vancomycin MICs greater than 2 mg/l.[63] However, daptomycin is inhibited by pulmonary surfactant, and should not be used to treat pneumonia.[77] Alternative agents for severe *S. aureus* infections include teicoplanin, tigecycline, quinupristin–dalfopristin and televancin. For MRSA-infections also the fifth generation cephalosporins ceftaroline and ceftobiprole may be considered.

Because of the severe complications of *S. aureus* bacteraemia and its propensity to relapse,[73] treatment with systemic antibiotic therapy for a minimum of 2 weeks is recommended, with a minimum of 1 week intravenous therapy.[78] Infections complicated by metastatic foci should be treated for 4-6 weeks; infections with non-removable intravascular foci (including infected thrombosis and endocarditis) should be treated with 6 weeks of intravenous, bactericidal therapy. *In vitro*, aminoglycosides act synergistically in *S. aureus* killing, and the addition of an aminoglycoside (most often gentamicin) may shorten the duration of fever and bacteraemia, although improved outcome with this combined therapy has not been demonstrated.

Linezolid or vancomycin should be added for anti-staphylococcal coverage in patients with a severe community acquired pneumonia suspected to be caused by CA-MRSA, and in patients developing HAP or VAP in institutions in which MRSA is a frequent nosocomial pathogen.

The optimal treatment for severe pneumonia caused by a PVL- producing *S. aureus* is still unclear. The UK based Health Protection Agency (HPA) recommends to initiate empiric combination of clindamycin, linezolid and rifampicin for severe pneumonia suspected to be caused by a PVL positive *S. aureus*, and to discontinue the linezolid if the cultured isolate is sensitive for clindamycin. The HPA also advises to add intravenous immunoglobulin's (IVIG) for severe cases and explicitly dissuades the use of beta-lactams.[79]

In treatment of prosthetic joint infections (with retention of the prosthesis) and prosthetic valve endocarditis, rifampicin is part of the antibiotic combination regimen, because of high penetration of this antibiotic in biofilms and its activity on slowly dividing bacteria.[80] *S. aureus* requires only a single mutation to become resistant against rifampicin and this may happen rapidly when the drug is used as monotherapy or with inadequate drug levels of the combination antibiotic. Therefore, it is recommended not to start rifampicin therapy before adequate levels of the other antibiotic have been secured and bacterial load reduction has been achieved, for instance after a minimum of two days therapy. Fusidic acid has been used as an alternative to rifampicin in the treatment of prosthetic valve endocarditis. Fusidic acid in combination with a second antibiotic agent (e.g. rifampin) has been used as an oral step down regimen in the treatment of bone infections, joints infections and prosthetic joint infections caused by methicillin-resistant staphylococci.[81]

Table 6. Characteristics of antimicrobial agents effective against *Staphylococcus aureus*

Agent	Mechanism of Action	Bacterial Effect	Principal adverse events
Isoxazolyl penicillins (oxacilline, cloxacillin, flucloxacillin, nafcillin)	Inhibiting cell wall synthesis	Bactericidal	Neurotoxicity and bone marrow suppression at high dosing, interstitial nephritis.
First generation cephalosporin (cefazolin, cephalexin)	Inhibiting cell wall synthesis	Bactericidal	Hepatitis, renal impairment.
Rifampin	Inhibiting RNA synthesis	Bactericidal	Elevated liver enzymes, discoloration of bodily fluids
Fusidic acid	Inhibiting protein synthesis	Bacteriostatic	GI side effects (nausea, diarrhea, discomfort). Elevated liver enzymes.
Vancomycin	Inhibiting cell wall assembly	Bactericidal	Nephrotoxicity; red man syndrome
Linezolid	Inhibiting protein synthesis (23S RNA at 50S ribosomal subunit)	Bacteriostatic	Peripheral and optic neuropathy, thrombocytopenia and anemia, lactic acidosis
Daptomycin	Membrane depolarization (Ca++ dependent)	Bactericidal	CK elevation, myopathy; peripheral neuropathy, rhabdomyolysis and eosinophilic pneumonia
Tigecycline	Inhibiting protein synthesis by binding to 30S ribosomal subunits	Bacteriostatic	GI side effects (nausea, vomiting)
Telavancin	Inhibiting formation of cell wall and depolarizes membrane	Bactericidal	GI side effects, nephrotoxicity, QT prolongation
Trimethoprim-sulfamethoxazole (TMP-SMZ)			GI side effects, rash, hematological suppression with longer use.
Quinolones (moxifloxacin, levofloxacin)	Inhibiting DNA synthesis by inhibiting the DNA gyrase and topoisomerase	Bactericidal	GI side effects (nausea, diarrhea). Tendon inflammation/ rupture, QT prolongation, irreversible peripheral neuropathy
Clindamycin	Inhibiting protein synthesis by binding to 50S ribosomal subunits	Bacteriostatic or bactericidal depending on drug concentration, infection site	GI side effects (diarrhea, CDAD, severe colitis)
Ceftaroline	Inhibiting cell wall synthesis	Bactericidal	Well tolerated (<5% incidence of diarrhea, nausea, rash)

Note: MSSA: methicillin susceptible *Staphylococcus aureus,* MRSA: methicillin resistant *Staphylococcus aureus*, RNA: ribonucleic acid, GI-tract: gastro-intestinal tract, MIC: minimal inhibitory concentration, VISA: vancomycin intermediate susceptible *S. aureus*, hVISA: heterogeneous vancomycin intermediate resistant *S. aureus,*

Advantages	Caveats
Well tolerated, rapidly bactericidal against MSSA	Not active against MRSA, low bioavailability (~50%) in oral formulations.
Well tolerated rapidly bactericidal against MSSA, formulations with high bioavailability available, broad spectrum.	Not active against MRSA, poor CSF penetration.
High bioavailability in oral formulation, good penetration in biofilms.	Inducible resistance, should not be given as monotherapy. numerous drug interactions
High bioavailability in oral formulation, high concentrations in bones and joints.	Not available in all counties, little experience with treating invasive infections.
Inexpensive, > 50 years of clinical experience	MIC >2mg/l associated with poor outcomes (VISA, hVISA, VRSA), nephrotoxicity may develop with longer durations of therapy
High bioavailability in oral formulation, good drug penetration into lung, recommended for treatment of pneumonia by MRSA	Bacteriostatic, not suitable for longer duration of therapy (maximum 28 days) due to serious adverse events with long-term use.
Rapidly bactericidal, effective for MRSA bloodstream infections and right-side endocarditis	Not suitable for treatment of pneumonia, due to inactivation by pulmonary surfactant; elevated vancomycin MIC have been associated with daptomycin resistance
	Bacteriostatic, GI side effects are common. higher risk of mortality than comparator agents. Low serum and ELF concentration
Rapidly bactericidal against MRSA, VISA, and VRSA; active against MRSA strains resistant to vancomycin, linezolid, and daptomycin	Nephrotoxicity, lower clinical outcomes in patients with reduced renal function, coagulation test interference.
Virtually no CA-MRSA resistance, oral formulation.	No data to support treatment of invasive infections.
High bioavailability oral formulation, broad spectrum.	Resistance may be induced with limited number of mutations; limited clinical experience in severe infections. Very broad spectrum, especially against Gram-negatives
Decreases toxin production, good bioavailability (90%) with oral formulation.	Inducible resistance, inadequate penetration into the CSF
Bactericidal, well tolerated	Most clinical data on bacterial skin and soft tissue infections

CK: creatine kinase, VRSA: vancomycin resistant *Staphylococcus aureus,* CA-MRSA: community associated methicillin resistant *Staphylococcus aureus,* DNA: deoxyribonucleic acid, CDAD: *Clostridium difficile* associated disease. CSF: cerebrospinal fluid.

MANAGEMENT OF COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

Vancomycin is usually the agent of choice in the treatment of CoNS infections, but when thorough laboratory testing indicates that a CoNS is methicillin susceptible, the isoxazolyl penicillins or first-generation cephalosporins are preferred. Other antibiotics which may be considered include clindamycin, teicoplanin, linezolid, daptomycin, co-trimoxazole, quinupristin–dalfopristin, ceftabiprole and ceftaroline. Similar to the treatment of prosthetic joints infections caused by *S. aureus*, rifampin is an integral part in the treatment of prosthetic joint infections with CoNS. CoNS are often multidrug-resistant, and therapy should be guided by the susceptibility test results.

AIM AND OUTLINE OF THIS THESIS

This thesis will focus on nosocomial transmission and resistance of *S. aureus* and CoNS. After the general introduction on *S. aureus* and coagulase-negative staphylococci, **part II** focuses on the nosocomial transmission capacity of different MRSA clones in the hospital setting. In **chapter 2** the nosocomial transmission capacity of livestock-associated MRSA (LA-MRSA) in the Dutch hospital setting is discussed. Previous findings have suggested that the nosocomial transmission capacity of LA-MRSA is lower than that of other MRSA genotypes. We performed a 6-month nationwide study to quantify the single-admission reproduction number, R_A, for LA-MRSA in 62 hospitals in the Netherlands and to compare this transmission capacity to previous estimates known from the literature. In **chapter 3** we quantified the risk of MRSA-transmission, in the absence of barrier precautions, in outpatient clinics and during short-term exposure in hospital wards. The risk of transmission after short-term exposure of MRSA carriers to a health-care setting is still unknown. In **Chapter 4** we quantified the transmission capacity by calculating R_A of community-associated MRSA (CA-MRSA) compared to health-care MRSA (HA-MRSA) in four Danish hospitals. The emergence of these so-called CA-MRSA clones has changed the epidemiology of MRSA infections worldwide. Despite obvious epidemiological differences, it is unknown whether differences in nosocomial transmissibility exist between CA-MRSA and HA-MRSA. The rapid emergence and high attack rates of CA-MRSA in the United States suggests that CA-MRSA strains are highly transmissible. In **Chapter 5** we investigate the molecular epidemiology of MRSA in thirteen European intensive care units. It is unknown to what extent the global changes in MRSA epidemiology affect ICU-populations in Europe. We determined the prevalence, acquisition rates and molecular epidemiology of MRSA in 13 intensive care units (ICUs) in eight European countries that participated in a prospective trial to control transmission of antibiotic resistance in ICUs (Mastering Hospital Antimicrobial Resistance in Europe (MOSAR) project).

Part III focuses on the acquisition and dynamics of mupirocin resistance in *S. aureus* and CoNS. Mupirocin is a topical antibiotic and the cornerstone of decolonization strategies of methicillin susceptible and methicillin resistant *S. aureus* (MSSA and MRSA). **Chapter 6** focuses on the clinical consequences of mupirocin resistance on decolonization success rates of *S. aureus* and MRSA, and on the associations between mupirocin use and the development of mupirocin resistance. In **chapter 7** we evaluated longitudinal trends in high-level mupirocin resistance in *S. aureus* and CoNS and its association with increasing mupirocin use in our hospital. In **chapter 8** we investigated the effects of a peri-operative universal decolonization strategy with topical mupirocin and chlorhexidine body washings, on developing mupirocin resistance in CoNS and *S. aureus*. Universal decolonization, as in treating everyone irrespective of *S. aureus* carrier ship status, is cost-effective and at least as effective in preventing *S. aureus* surgical site infections. However, the extensive use of mupirocin may facilitate emergence of mupirocin resistance in *S. aureus* and CoNS. In **chapter 9** we compare the efficacy, cost-effectiveness and the risks of developing resistance for two different peri-operative *S. aureus* decolonization strategies: targeted screening and decolonization versus universal decolonization, i.e. treating everyone irrespective of *S. aureus* carrier ship status. We use a deterministic mathematical model to explore the dynamics of mupirocin resistance within a hospital ward and identify parameters that are important drivers for mupirocin resistance in *S. aureus*.

In **part IV** and **chapter 10** we discuss the management of intravascular catheters colonized with *S. aureus*. Previous studies in tertiary care hospitals identified *S. aureus* colonization of intravascular catheters as a strong predictor of subsequent *S. aureus* bacteremia (SAB), even in the absence of clinical signs of systemic infection. We wanted to corroborate the validity of these findings in non-university hospitals. Subsequently, we performed a systematic review and meta-analysis of all observational studies evaluating the effect of antibiotic therapy for *S. aureus* intravascular catheter tip colonization.

A discussion and synthesis of this thesis is provided in **chapter 11.**

REFERENCES

1. Turner RD, Ratcliffe EC, Wheeler R, Golestanian R, Hobbs JK, Foster SJ. Peptidoglycan architecture can specify division planes in staphylococcus aureus. *Nat Commun*. 2010;1:26.
2. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in staphylococcus aureus infections. *Lancet Infect Dis*. 2005;5(12):751-762.
3. Grundmann H, Aanensen DM, van den Wijngaard CC, et al. Geographic distribution of staphylococcus aureus causing invasive infections in europe: A molecular-epidemiological analysis. *PLoS Med*. 2010;7(1):e1000215.
4. Miko BA, Hafer CA, Lee CJ, et al. Molecular characterization of methicillin-susceptible staphylococcus aureus clinical isolates in the united states, 2004 to 2010. *J Clin Microbiol*. 2013;51(3):874-879.

5. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant staphylococcus aureus (MRSA). *Proc Natl Acad Sci U S A*. 2002;99(11):7687-7692.
6. Ammerlaan HS, Harbarth S, Buiting AG, et al. Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin Infect Dis*. 2013;56(6):798-805.
7. National Nosocomial Infections Surveillance System. National nosocomial infections surveillance (NNIS) system report, data summary from january 1992 through june 2004, issued october 2004. *Am J Infect Control*. 2004;32(8):470-485.
8. Dantes R, Mu Y, Belflower R, et al. National burden of invasive methicillin-resistant staphylococcus aureus infections, united states, 2011. *JAMA Intern Med*. 2013;173(21):1970-1978.
9. Perl TM, Cullen JJ, Wenzel RP, et al. Intranasal mupirocin to prevent postoperative staphylococcus aureus infections. *N Engl J Med*. 2002;346(24):1871-1877.
10. Muder RR, Brennen C, Rihs JD, et al. Isolation of staphylococcus aureus from the urinary tract: Association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. *Clin Infect Dis*. 2006;42(1):46-50.
11. Squier C, Rihs JD, Risa KJ, et al. Staphylococcus aureus rectal carriage and its association with infections in patients in a surgical intensive care unit and a liver transplant unit. *Infect Control Hosp Epidemiol*. 2002;23(9):495-501.
12. Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. *Lancet*. 2004;364(9435):703-705.
13. Tenover FC, Tickler IA, Goering RV, et al. Characterization of nasal and blood culture isolates of methicillin-resistant staphylococcus aureus from patients in united states hospitals. *Antimicrob Agents Chemother*. 2012;56(3):1324-1330.
14. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant staphylococcus aureus. *Lancet*. 2010;375(9725):1557-1568.
15. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus. *Emerg Infect Dis*. 2013;19(11):1797-1802.
16. Selton-Suty C, Celard M, Le Moing V, et al. Preeminence of staphylococcus aureus in infective endocarditis: A 1-year population-based survey. *Clin Infect Dis*. 2012;54(9):1230-1239.
17. Nouwen JL, van Belkum A, de Marie S, et al. Clonal expansion of staphylococcus epidermidis strains causing hickman catheter-related infections in a hemato-oncologic department. *J Clin Microbiol*. 1998;36(9):2696-2702.
18. Westerlund B, Korhonen TK. Bacterial proteins binding to the mammalian extracellular matrix. *Mol Microbiol*. 1993;9(4):687-694.
19. Bowden MG, Heuck AP, Ponnuraj K, et al. Evidence for the “dock, lock, and latch” ligand binding mechanism of the staphylococcal microbial surface component recognizing adhesive matrix molecules (MSCRAMM) SdrG. *J Biol Chem*. 2008;283(1):638-647.
20. Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A. Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. *J Immunol*. 2004;172(2):1169-1176.
21. Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F. Inactivation of the dlt operon in staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. *J Biol Chem*. 1999;274(13):8405-8410.
22. Walport MJ. Complement. first of two parts. *N Engl J Med*. 2001;344(14):1058-1066.
23. Walport MJ. Complement. second of two parts. *N Engl J Med*. 2001;344(15):1140-1144.

24. Rooijakkers SH, Milder FJ, Bardoel BW, Ruyken M, van Strijp JA, Gros P. Staphylococcal complement inhibitor: Structure and active sites. *J Immunol*. 2007;179(5):2989-2998.
25. Langley R, Wines B, Willoughby N, Basu I, Proft T, Fraser JD. The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-fc alpha RI binding and serum killing of bacteria. *J Immunol*. 2005;174(5):2926-2933.
26. Jongerius I, von Kockritz-Blickwede M, Horsburgh MJ, Ruyken M, Nizet V, Rooijakkers SH. Staphylococcus aureus virulence is enhanced by secreted factors that block innate immune defenses. *J Innate Immun*. 2012;4(3):301-311.
27. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005;3(12):948-958.
28. Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med*. 2007;13(12):1510-1514.
29. Ley K. Integration of inflammatory signals by rolling neutrophils. *Immunol Rev*. 2002;186:8-18.
30. Bestebroer J, Poppelier MJ, Ulfman LH, et al. Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. *Blood*. 2007;109(7):2936-2943.
31. Laarman AJ, Mijnheer G, Mootz JM, et al. Staphylococcus aureus staphopain A inhibits CXCR2-dependent neutrophil activation and chemotaxis. *EMBO J*. 2012;31(17):3607-3619.
32. Bardoel BW, Vos R, Bouman T, et al. Evasion of toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. *J Mol Med (Berl)*. 2012;90(10):1109-1120.
33. Spaan AN, Surewaard BG, Nijland R, van Strijp JA. Neutrophils versus staphylococcus aureus: A biological tug of war. *Annu Rev Microbiol*. 2013;67:629-650.
34. Ko YP, Kuipers A, Freitag CM, et al. Phagocytosis escape by a staphylococcus aureus protein that connects complement and coagulation proteins at the bacterial surface. *PLoS Pathog*. 2013;9(12):e1003816.
35. O'Riordan K, Lee JC. Staphylococcus aureus capsular polysaccharides. *Clin Microbiol Rev*. 2004;17(1):218-234.
36. Liu GY, Essex A, Buchanan JT, et al. Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med*. 2005;202(2):209-215.
37. Spaan AN, Henry T, van Rooijen WJ, et al. The staphylococcal toxin panton-valentine leukocidin targets human C5a receptors. *Cell Host Microbe*. 2013;13(5):584-594.
38. Surewaard BG, Nijland R, Spaan AN, Kruijtzer JA, de Haas CJ, van Strijp JA. Inactivation of staphylococcal phenol soluble modulins by serum lipoprotein particles. *PLoS Pathog*. 2012;8(3):e1002606.
39. Nakamura Y, Oscherwitz J, Cease KB, et al. Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. *Nature*. 2013;503(7476):397-401.
40. Kawabata S, Morita T, Miyata T, Iwanaga S, Igarashi H. Isolation and characterization of staphylocoagulase chymotryptic fragment. localization of the procoagulant- and prothrombin-binding domain of this protein. *J Biol Chem*. 1986;261(3):1427-1433.
41. de Haas CJ, Veldkamp KE, Peschel A, et al. Chemotaxis inhibitory protein of staphylococcus aureus, a bacterial antiinflammatory agent. *J Exp Med*. 2004;199(5):687-695.
42. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol Microbiol*. 2003;48(6):1429-1449.
43. Surewaard BG, de Haas CJ, Vervoort F, et al. Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell Microbiol*. 2013;15(8):1427-1437.
44. Voyich JM, Vuong C, DeWald M, et al. The SaeR/S gene regulatory system is essential for innate immune evasion by staphylococcus aureus. *J Infect Dis*. 2009;199(11):1698-1706.

45. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis*. 2002;2(11):677-685. doi: S1473309902004383 [pii].
46. Johnson AP, Davies J, Guy R, et al. Mandatory surveillance of methicillin-resistant staphylococcus aureus (MRSA) bacteraemia in england: The first 10 years. *J Antimicrob Chemother*. 2012;67(4):802-809.
47. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of staphylococcus aureus. *N Engl J Med*. 2010;362(1):9-17.
48. Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ. Eradication of methicillin-resistant staphylococcus aureus carriage: A systematic review. *Clin Infect Dis*. 2009;48(7):922-930.
49. Simor AE. Staphylococcal decolonisation: An effective strategy for prevention of infection? *Lancet Infect Dis*. 2011;11(12):952-962.
50. Ogston A. Report upon micro-organisms in surgical diseases. *Br Med J*. 1881;1(1054):369.b2-375.
51. Blair EB, Emerson JS, Tull AH. A new medium, salt mannitol plasma agar, for the isolation of staphylococcus aureus. *Am J Clin Pathol*. 1967;47(1):30-39.
52. Loonen AJ, Jansz AR, Bergland JN, Valkenburg M, Wolffs PF, van den Brule AJ. Comparative study using phenotypic, genotypic, and proteomics methods for identification of coagulase-negative staphylococci. *J Clin Microbiol*. 2012;50(4):1437-1439.
53. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother*. 1999;43(12):2823-2830.
54. Garcia-Alvarez L, Holden MT, Lindsay H, et al. Meticillin-resistant staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and denmark: A descriptive study. *Lancet Infect Dis*. 2011;11(8):595-603.
55. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant staphylococcus aureus. *Trends Microbiol*. 2014;22(1):42-47.
56. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant staphylococcus aureus (MRSA): A disk diffusion method with cefoxitin and moxalactam, the vitek 2 system, and the MRSA-screen latex agglutination test. *J Clin Microbiol*. 2002;40(8):2766-2771.
57. Nahimana I, Francioli P, Blanc DS. Evaluation of three chromogenic media (MRSA-ID, MRSA-select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant staphylococcus aureus. *Clin Microbiol Infect*. 2006;12(12):1168-1174.
58. Tenover FC, Moellering RC,Jr. The rationale for revising the clinical and laboratory standards institute vancomycin minimal inhibitory concentration interpretive criteria for staphylococcus aureus. *Clin Infect Dis*. 2007;44(9):1208-1215.
59. Cui L, Iwamoto A, Lian JQ, et al. Novel mechanism of antibiotic resistance originating in vancomycin-intermediate staphylococcus aureus. *Antimicrob Agents Chemother*. 2006;50(2):428-438.
60. Boyle-Vavra S, Berke SK, Lee JC, Daum RS. Reversion of the glycopeptide resistance phenotype in staphylococcus aureus clinical isolates. *Antimicrob Agents Chemother*. 2000;44(2):272-277.
61. Holmes NE, Turnidge JD, Munckhof WJ, et al. Antibiotic choice may not explain poorer outcomes in patients with staphylococcus aureus bacteremia and high vancomycin minimum inhibitory concentrations. *J Infect Dis*. 2011;204(3):340-347.
62. Maor Y, Hagin M, Belausov N, Keller N, Ben-David D, Rahav G. Clinical features of heteroresistant vancomycin-intermediate staphylococcus aureus bacteremia versus those of methicillin-resistant S. aureus bacteremia. *J Infect Dis*. 2009;199(5):619-624.
63. Fowler VG,Jr, Boucher HW, Corey GR, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by staphylococcus aureus. *N Engl J Med*. 2006;355(7):653-665.

64. Gu B, Kelesidis T, Tsiodras S, Hindler J, Humphries RM. The emerging problem of linezolid-resistant staphylococcus. *J Antimicrob Chemother*. 2013;68(1):4-11.
65. Gordon NC, Price JR, Cole K, et al. Prediction of staphylococcus aureus antimicrobial resistance by whole-genome sequencing. *J Clin Microbiol*. 2014;52(4):1182-1191.
66. Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A*. 1998;95(6):3140-3145.
67. Schouls LM, Spalburg EC, van Luit M, et al. Multiple-locus variable number tandem repeat analysis of staphylococcus aureus: Comparison with pulsed-field gel electrophoresis and spa-typing. *PLoS One*. 2009;4(4):e5082.
68. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-5448.
69. Price JR, Golubchik T, Cole K, et al. Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of staphylococcus aureus in an intensive care unit. *Clin Infect Dis*. 2014;58(5):609-618.
70. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A. Bacterial whole genome sequencing revisited: Portable, scalable and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol*. 2014.
71. Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. *Clin Microbiol Rev*. 2013;26(3):422-447.
72. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2006-2007. *Infect Control Hosp Epidemiol*. 2008;29(11):996-1011.
73. Fowler VG, Jr, Olsen MK, Corey GR, et al. Clinical identifiers of complicated staphylococcus aureus bacteremia. *Arch Intern Med*. 2003;163(17):2066-2072.
74. Fowler VG, Jr, Justice A, Moore C, et al. Risk factors for hematogenous complications of intravascular catheter-associated staphylococcus aureus bacteremia. *Clin Infect Dis*. 2005;40(5):695-703.
75. Fortun J, Perez-Molina JA, Anon MT, Martinez-Beltran J, Loza E, Guerrero A. Right-sided endocarditis caused by staphylococcus aureus in drug abusers. *Antimicrob Agents Chemother*. 1995;39(2):525-528.
76. van Hal SJ, Paterson DL, Lodise TP. Systematic review and meta-analysis of vancomycin-induced nephrotoxicity associated with dosing schedules that maintain troughs between 15 and 20 milligrams per liter. *Antimicrob Agents Chemother*. 2013;57(2):734-744.
77. Silverman JA, Mortin LI, Vanpraagh AD, Li T, Alder J. Inhibition of daptomycin by pulmonary surfactant: In vitro modeling and clinical impact. *J Infect Dis*. 2005;191(12):2149-2152.
78. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the infectious diseases society of america. *Clin Infect Dis*. 2009;49(1):1-45.
79. Health Protection Agency (HPA). Guidance on the diagnosis and management of PVL-associated staphylococcus aureus infections (PVL-SA) in England. November 2008, revised 2010.
80. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med*. 2004;351(16):1645-1654.
81. Aboltins CA, Page MA, Buising KL, et al. Treatment of staphylococcal prosthetic joint infections with debridement, prosthesis retention and oral rifampicin and fusidic acid. *Clin Microbiol Infect*. 2007;13(6):586-591.

part II

NOSOCOMIAL TRANSMISSION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

two

TRANSMISSIBILITY OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

David J. Hetem, Martin C.J. Bootsma, Annet Troelstra, Marc J.M. Bonten

Emerging Infectious Diseases, 2013

ABSTRACT

Previous findings have suggested that the nosocomial transmission capacity of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is lower than that of other MRSA genotypes. We therefore performed a 6-month (June 1–November 30, 2011) nationwide study to quantify the single-admission reproduction number, R_A, for LA-MRSA in 62 hospitals in the Netherlands and to compare this transmission capacity to previous estimates. We used *spa* typing for genotyping. Quantification of R_A was based on a mathematical model incorporating outbreak sizes, detection rates, and length of hospital stay. There were 141 index cases, 40 (28%) of which were LA-MRSA. Contact screening of 2,101 patients and 7,260 health care workers identified 18 outbreaks (2 LA-MRSA) and 47 secondary cases (3 LA-MRSA). R_{A-} values indicated that transmissibility of LA-MRSA is 4.4 times lower than that of other MRSA (not associated with livestock).

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of nosocomial infections and leads to considerable illness, death, and health care costs.[1,2] The worldwide epidemiology of MRSA has changed as MRSA originating in the community has increased. These community-associated MRSA (CA-MRSA) strains are replacing their hospital-associated counterparts in hospitals in the United States; the major dominant clone is MRSA strain USA300.[3] In recent years, another MRSA clone, which originated in the community and is associated with exposure to livestock, has emerged in different countries worldwide, including the United States.[4] Even more worrying, countries with a historically low prevalence of MRSA, like the Netherlands and Denmark, have seen an increase in livestock-associated MRSA (LA-MRSA), belonging to clonal complex 398.[5] In the Netherlands, LA-MRSA accounted for 39% of all new MRSA isolated in 2011.[6] Yet almost all isolates have been detected through screening, and in 2009, nine infections were caused by MRSA sequence type 398.[7] Invasive infections caused by LA-MRSA include endocarditis, osteomyelitis, and ventilator-associated pneumonia.[8,9]

It has been suggested that in the Netherlands, this MRSA genotype has a lower capacity than other genotypes for nosocomial transmission.[10,11] The lower transmission rates might result from differences in human host characteristics or from a lack of pathogen adaptation to the human host, which could change over time.[12] In a previous study in the Netherlands in 2005, we quantified the transmission capacity, expressed as the single-admission reproduction number per hospital admission, R_A, and obtained values of 0.16 for LA-MRSA and 0.68 and 0.98 for MRSA not associated with livestock (hereafter referred to as other MRSA).[10] We therefore performed a nationwide study to quantify R_A for LA-MRSA in hospitals in the Netherlands and to compare this transmission capacity to our previous estimates.

METHODS

Data Collection

Medical microbiologists and infection control practitioners in all 91 hospitals in the Netherlands were contacted and asked to collect data concerning MRSA outbreaks and the results of subsequent contact screening retrospectively during June–August 2011 and prospectively during September–November 2011. A standardized Web page was used for data collection. An index case-patient was defined as a hospitalized patient colonized or infected with MRSA and treated without use of barrier precautions. Age, sex, and number of days hospitalized from MRSA detection through discharge were obtained. According to the guidelines in the Netherlands, identification of a MRSA index case-patient initiates contact screening among contact patients and health care workers (HCWs).[13] The numbers of screened patients and HCWs and the number of secondarily colonized patients and HCWs were obtained. A secondary case-patient was defined as a patient with MRSA with a *spa* type

identical or related to that from the index case-patient, detected during contact screening of a patient or HCW. Newly identified MRSA carriers with MRSA *spa* types that were unrelated to that of an index case-patient were considered incidental findings. The study was approved by the medical research ethics committee of the University Medical Center Utrecht.

MRSA Genotyping

For all MRSA isolates, single-locus DNA sequencing of the repeat region of *Staphylococcus* protein A gene (*spa* typing) was performed by the national reference laboratory of the Netherlands (National Institute for Public Health and the Environment [RIVM]), as described,[14] by use of the Ridom StaphType program (www.ridom.de) to allocate *spa* types. MRSA isolates were considered to be associated with livestock if they had a livestock-associated *spa* type: *t011, t034, t108, t567, t571, t588, t753, 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*, or *t3208*.[5,15,16] All other *spa* types were considered to not be associated with livestock. To identify potentially unknown livestock *spa* types, we used Bionumerics 5.1 (Applied-Maths, Sint Maartens-Latem, Belgium) to create a *spa*-based minimal spanning tree of *spa* types considered livestock-associated and the *spa* types of index cases (Technical Appendix Figure). Genes encoding for Panton-Valentine leukocidin (PVL), *LukS-PV*, and *LukF-PV* were identified by the reference laboratory, as described.[17]

Model

To estimate the strain-specific transmission capacity R_A value, we used a previously described mathematical model based on queueing theory.[18] R_A is defined as the average number of secondary cases caused by 1 primary case (the index case) when other patients are susceptible during a single hospital admission of the primary case-patient.[19] In this model, 3 rates determine the spread of MRSA in the hospital setting: the rate at which the MRSA strain spreads, the rate at which MRSA colonization of a patient is detected (e.g., microbiological cultures), and the rate at which a colonized patient can no longer be detected. The model predicts that the distribution of the number of patients colonized at the time of detection of the index case is geometrically distributed. The parameter of the geometric distribution of detected outbreak sizes was determined by using maximum-likelihood estimations. Small detected outbreak sizes could correspond to either low transmission potential or high detection rate.

Patients with MRSA remain colonized during their hospital stay; therefore, the infectious period ends at the time of discharge. Genotype-specific discharge rates were calculated from admission and discharge data for index case-patients admitted to participating hospitals during the study period. The detection rate was based on all blood, respiratory tract, and wound cultures conducted during 2011 at the University Medical Center Utrecht. The upper detection limit consists of all these cultures divided by the total number of patient days in 2011. By combining the detection and discharge rate with the parameter of

geometric distribution, we could calculate R_A. Details about the model are included in the online Technical Appendix.

Statistical Analyses

Categorical variables were assessed 2-sided by using χ^2 or Fisher exact tests, as appropriate; a cutoff value of $p < 0.05$ was applied for significance. Continuous variables were analyzed by using the Mann-Whitney U test. Confidence intervals were calculated by using the profile-likelihood method. To test whether our assumption of a geometrical distribution of the detected outbreak sizes is justified by the data, we performed the Anderson-Darling goodness-of-fit test. Data were analyzed by using SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Further details about the statistical methods used are included in the online Technical Appendix.

RESULTS

A total of 62 (69%) of the 91 hospitals in the Netherlands participated in the study, yielding data for 372 months of MRSA policy. During the 6-month study period, 158 MRSA index case-patients were identified in 57 hospitals, and none were identified in the other 5 hospitals. These numbers imply that, on average, in each hospital an index case was detected every 2.5 months. Two index case-patients were excluded because subsequent contact screening was not performed, and 15 index case-patients were excluded because barrier precautions were implemented on the day of admission. For these 15 index case-patients, contact screenings of 55 patients and 293 HCWs had identified 1 MRSA-colonized HCW with an unrelated MRSA genotype. For the remaining 141 index case-patients, 9,361 contacts (2,101 patients and 7,260 HCWs) were screened.

In total, 65 *spa* types were identified among the 141 index cases; the most common were t011 (n = 25 [18%]), t008 (n = 12 [9%]), and t002 (n = 7 [5%]). A total of 40 (29%) isolates had *spa* types indicative of LA-MRSA; the most prevalent were t011 (n = 25), t034 (n = 6), and t108 (n = 6) (Table 1).

Luk-PV genes, indicative of PVL, were detected in 24 (18%) of 131 isolates investigated, all categorized as not being LA-MRSA strains. Among 12 MRSA *spa* type t008 isolates, PVL positivity was detected in 8 (67%) (Table 1), and among 10 (7%) MRSA isolates, the presence of PVL was undetermined.

Mean age among all index case-patients was 53 years. Among patients with LA-MRSA and other MRSA genotypes, no significant differences were found except for sex (Table 2). Among index case-patients with LA-MRSA genotypes, 83% were male, compared with 56% case-patients with other MRSA ($p = 0.004$). No statistically significant differences were found in length of hospital stay ($p = 0.222$) and number of days in hospital without barrier precautions ($p = 0.503$) between index case-patients with LA-MRSA and patients with other MRSA genotypes (Table 2).

Table 1. Genotypes of methicillin-resistant *Staphylococcus aureus* from index and secondary case-patients

spa-type	PVL	Index cases n= 141	Number of outbreaks n= 18	Secondary cases n= 47
LA-MRSA				
t011	0 / 24	25	1	2 (4%)
t034	0 / 5	6	0	0
t108	0 / 5	6	1	1 (2%)
t899	0 / 2	2	0	0
t2330	0 / 1	1	0	0
Other MRSA				
t008	8 / 12 (67%)	12	0	0
t002	1 / 7 (14%)	7	2	4 (2%)
t032	0 / 5	5	1	6 (13%)
t064	0 / 5	5	1	5 (11%)
t1081	0 / 3	5	3	14 (31%)
t688	0 / 3	4	0	0
t038	1 / 3 (33%)	3	1	1 (2%)
t267	0 / 3	3	0	0
t001	0 / 1	2	0	0
t018	0 / 2	2	0	0
t179	0 / 2	2	1	1 (2%)
t447	0 / 2	2	1	1 (2%)
t1430	0 / 2	2	0	0
t1469	0 / 2	2	0	0
Singletons	14 / 42 (33%) [a]	45	6 [b]	12 (24%)

Note: PVL: Panton-Valentine leucocidin, LA-MRSA: livestock-associated methicillin resistant *Staphylococcus aureus*. [a] PVL positive: t022, t040, t044, t054, t131, t311, t318, t437, t657, t690, t791, t852, t2815, t3523, t7277; [b] *Spa* types causing outbreaks : t003, t088, t311, t1399, t7277, t9634.

Among 141 post exposure screenings, MRSA carriers were identified for 18 (13%) case-patients, yielding 39 newly identified colonized patients and 34 newly identified colonized HCWs with MRSA. Screening of index case-patients with LA-MRSA identified 15 (21%) carriers, and screening of index case-patients with other MRSA identified 58 (79%) carriers. Of these 73 MRSA carriers, 47 (64%) were colonized with a MRSA *spa* type that was identical to that of the corresponding index case-patient; 3 patients had *spa* types matching those of 2 index case-patients with LA-MRSA, and 44 had *spa* types matching those of 16 index case-patients with other MRSA. Transmission of MRSA (i.e., outbreaks) was documented for 18 index patients; the largest outbreak consisted of 12 secondary cases (8 patients and

Table 2. Characteristics of index case-patients with LA-MRSA and other MRSA genotypes

Characteristic	LA-MRSA n= 40	Other MRSA n= 101	*P* value
R_A (95% CI)	0.12 (0.03-0.30)	0.52 (0.38-0.69)	NA
Age (years)	56	52	0.337
Male, no. (%)	33 (83%)	57 (56%)	0.004
Length of stay (days, median)	13	10	0.222
Days not in isolation (days, median)	5	6	0.503

Note: LA-MRSA : livestock-associated methicillin-resistant *Staphylococcus aureus*. NA : not applicable.

4 HCWs, *spa* type t1081), and most outbreaks (11 [61%] of 18) consisted of only 1 secondary case (Figure 1). Contact screening for 1 index case-patient with LA-MRSA (t011) revealed 1 outbreak consisting of 3 patients with a MRSA genotype (t067) that was not LA-MRSA. These newly identified cases of MRSA carriage were considered to be not associated with the index case with an LA-MRSA genotype.

During 2011, a total of 6,819 blood, 4,828 respiratory tract, and 1,132 wound cultures were performed. For the upper limit of detection, we used only 1 culture per patient per day, yielding 11,903 relevant cultures, divided by the number of patient-days (241,319) (Technical Appendix Table A).

The ratio between detection and discharge rates did not differ much between patients with LA-MRSA and other MRSA (Technical Appendix Table A). The parameter for geometric distribution for LA-MRSA and other MRSA is also provided in the online Technical Appendix. There was no reason to reject the hypothesis of a geometrically distributed outbreak size for LA-MRSA; but the hypothesis was rejected for other MRSA ($p<0.05$).

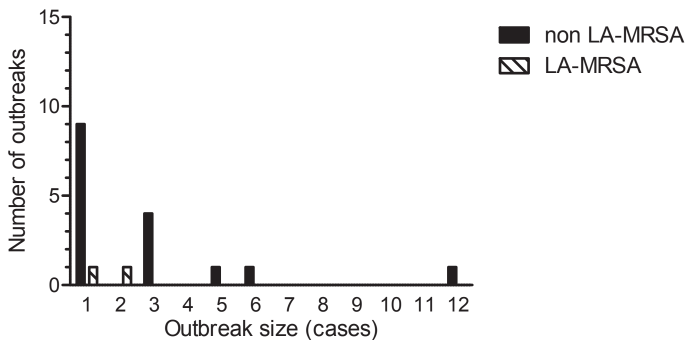


Figure 1. Number of outbreaks and outbreak sizes (number of cases, excluding the index case). LA-MRSA, livestock-associated methicillin-resistant*Staphylococcus aureus*; other MRSA, MRSA not associated with livestock.

Based on the genotype-specific ratio between detection and discharge rates, R_A values were 0.43 (95% CI 0.32–0.56), 0.12 (95% CI 0.03–0.31), and 0.52 (95% CI 0.38–0.69) for all 27 genotypes, LA-MRSA, and other MRSA, respectively. According to these R_A-values, the transmissibility of LA-MRSA was considered 4.4 times lower than that of other MRSA (0.12/0.52). The R_A value for PVL-positive strains was 0.31 (95% CI 0.14–0.58).

DISCUSSION

Using data from 62 hospitals in the Netherlands, comprising 372 months of MRSA policy, we determined that livestock-associated MRSA genotypes, compared with other MRSA genotypes, are 4.4 times less likely to spread in the hospital. Our findings in this study add substantial knowledge to findings from our previous study of hospitals in the Netherlands in 2005.[10,11] The current study included a larger cohort of hospitals and genotyping of all isolates. In our previous study, we compared *sma*I non-typeable MRSA to other MRSA genotypes without further genotyping. The genotyping demonstrates the heterogeneity in index cases with MRSA not associated with livestock. Moreover, in the current study, we collected more detailed patient information, such as admission and discharge dates and the number of days that index and secondary case-patients were treated without barrier precautions, which enabled more precise estimation of parameters. Absence of significant differences in age, length of hospital stay, or number of days not spent in isolation between index case-patients with LA-MRSA and those with other MRSA reduces the possibility that the differences in transmission capacity resulted from differences in patient characteristics. The only difference was that LA-MRSA index case-patients were more likely to be male, reflecting sex distributions among pig farmers and veal calf farmers.

For this study, we made several assumptions. First, no differentiation was made between patients and HCWs. Both are at risk for colonization with MRSA; however, infectious period and infectivity may differ. Second, all carriers were assumed to be equally infectious; whereas, super spreaders could play a major role in the transmission of MRSA in certain outbreaks. The consequences of these assumptions have been discussed in detail elsewhere.[10]

This study has several limitations. For this model to work, MRSA outbreaks must be rare and rigorous screening must be performed after the identification of an index case. If multiple outbreaks of the same genotype occur on the same ward, R_A would be an overestimation. Here, *spa* typing was used to identify cases of transmission between index and secondary case-patients. Among LA-MRSA, 63% were *spa* type t011; whereas, other MRSA consist of many different *spa* types. The high prevalence of LA-MRSA in pig-dense areas combined with the homogeneity of *spa* types could lead to an actual overestimation of these transmission events (and the estimated R_A values of LA-MRSA).

LA-MRSA comprise a well-defined set of *spa* types, most commonly t011, t034, and t108; whereas, other MRSA comprise a highly heterogeneous group with hospital-

associated genotypes and PVL-positive, community-associated genotypes.[20] Almost 25% of all other MRSA were PVL positive, which is considered a characteristic of community-associated MRSA. Although 25% seems high, the actual incidence of index case-patients with PVL-positive MRSA was 24 in 379 hospital months, comprising an average of 1 index case per 16 months per hospital. In contrast to LA-MRSA and hospital-associated MRSA, there are no established risk factors in the Netherlands for colonization with CA-MRSA, and unknown carriers of these genotypes will not be screened when admitted to hospital.[13] Although another study from the Netherlands reported a high number of PVL-positive isolates in MRSA-colonized patients without risk factors as described in the national guidelines,[13,21] our findings demonstrate that PVL-positive strains do not constitute a major risk for health care settings in the Netherlands because the introduction rate and the R_A in the absence of barrier precautions (R_A for PVL-positive strains 0.31, 95% CI 0.14–0.58) are low. Nevertheless, if admission rates increase, outbreaks could emerge despite R_A values <1.[19]

spa type t1081 was associated with the highest number of outbreaks and with most secondary cases. This *spa* type has also been associated with outbreaks in nursing homes across the Netherlands. For s*pa* type t1081, the MIC for cefoxitin (data not shown) is low (3 mg/L [range 3–8 mg/L]), hampering laboratory detection during routine procedures, which might have contributed to the high number of secondary cases found with this *spa* type.

Whole-genome analyses of multiple sequence type 398 *S. aureus* strains suggests that LA-MRSA originated from methicillin-susceptible *S. aureus* that crossed species barriers from humans to livestock, where it acquired resistance traits.[22] It has been hypothesized that the transition from humans to animals was associated with the loss of several human immune evasion genes, carried on phage φSa3, which may prevent human niche adaptation of LA-MRSA.[23] Whether this loss is associated with the lower R_A remains to be determined.

The epidemiology of CA-MRSA in Europe differs markedly from that in the United States; >50% of community-acquired *S. aureus* infections in Europe are caused by a few PVL-positive clones.[24] There is a paucity of data on the nosocomial transmission capacity of CA-MRSA. In hospitals in the Netherlands, though, the estimated R_A of CA-MRSA, consisting of a heterogeneous group of genotypes, was estimated to be 0.07 (95% CI 0.00–0.28),[25] and in the hospitals participating in the present study, the R_A value of PVL MRSA strains was 0.31, 95% CI 0.14–0.58. The differences between Europe and the United States regarding the epidemiology of PVL-positive CA-MRSA, therefore, remain unexplained.

Current guidelines in the Netherlands recommend MRSA screening for all patients with professional exposure to livestock, and many hospitals treat such patients in isolation while screening results are pending (i.e., preemptive isolation). In a previous multicenter study in the Netherlands, we demonstrated the cost-effectiveness and safety of not preemptively isolating patients when using rapid diagnostic testing.[26] That evaluation included all MRSA genotypes: LA-MRSA and other MRSA. The confirmation of the lower transmissibility of

LA-MRSA (in combination with the low R_A value) and the results of the previous study provide evidence that preemptive isolation may not be necessary for LA-MRSA, which would substantially enhance the feasibility of this highly successful infection control policy.

ACKNOWLEDGMENTS

We thank all medical microbiologists and infection prevention practitioners who participated in this study.

FUNDING

This work was supported by The Netherlands Organization of Scientific Research (NWO-VICI 918.76.611 to M.J.M.B.).

REFERENCES

1. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant staphylococcus aureus and escherichia coli bacteremia: Estimating the burden of antibiotic resistance in europe. *PLoS Med*. 2011;8(10):e1001104.
2. Kaye KS, Anderson DJ, Choi Y, Link K, Thacker P, Sexton DJ. The deadly toll of invasive methicillin-resistant staphylococcus aureus infection in community hospitals. *Clin Infect Dis*. 2008;46(10):1568-1577.
3. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis*. 2008;46(6):787-794.
4. Smith TC, Male MJ, Harper AL, et al. Methicillin-resistant staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. *PLoS One*. 2009;4(1):e4258.
5. van Cleef BA, Monnet DL, Voss A, et al. Livestock-associated methicillin-resistant staphylococcus aureus in humans, europe. *Emerg Infect Dis*. 2011;17(3):502-505.
6. Stichting Werkgroep Antibiotica Beleid. NethMap 2012: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. http://www.swab.nl/swab/cms3.nsf/uploads/E8426668DC9BC944C1257A24006252DE/$FILE/Nethmap_Maran_2012.pdf. Accessed 07/04, 2015.
7. van Cleef BA, van Benthem BH, Haenen AP, Bosch T, Monen J, Kluytmans JA. Low incidence of livestock-associated methicillin-resistant staphylococcus aureus bacteraemia in the netherlands in 2009. *PLoS One*. 2013;8(8):e73096.
8. Mammina C, Cala C, Plano MR, et al. Ventilator-associated pneumonia and MRSA ST398, italy. *Emerg Infect Dis*. 2010;16(4):730-731.
9. Ekkelenkamp MB, Sekkat M, Carpaij N, Troelstra A, Bonten MJ. Endocarditis due to meticillin-resistant staphylococcus aureus originating from pigs. *Ned Tijdschr Geneeskd*. 2006;150(44):2442-2447.
10. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.

11. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus (ST398) in dutch hospitals. *Clin Microbiol Infect*. 2011;17(2):316-319.
12. Valentin-Domelier AS, Girard M, Bertrand X, et al. Methicillin-susceptible ST398 staphylococcus aureus responsible for bloodstream infections: An emerging human-adapted subclone? *PLoS One*. 2011;6(12):e28369.
13. Infection Prevention Working Party. MRSA hospital guideline. http://www.wip.nl/UK/free_content/Richtlijnen/110530%20MRSA%20hospital%20def.pdf. Accessed 05/04, 2015.
14. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-5448.
15. Huijsdens XW, Bosch T, van Santen-Verheuvel MG, et al. Molecular characterisation of PFGE non-typable methicillin-resistant staphylococcus aureus in the netherlands, 2007. *Euro Surveill*. 2009;14(38):19335.
16. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. Nasal colonization of humans with methicillin-resistant staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. *PLoS One*. 2009;4(8):e6800.
17. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of panton-valentine leukocidin-producing staphylococcus aureus in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29(5):1128-1132.
18. Trapman P, Bootsma MC. A useful relationship between epidemiology and queueing theory: The distribution of the number of infectives at the moment of the first detection. *Math Biosci*. 2009;219(1):15-22.
19. Cooper BS, Medley GF, Stone SP, et al. Methicillin-resistant staphylococcus aureus in hospitals and the community: Stealth dynamics and control catastrophes. *Proc Natl Acad Sci U S A*. 2004;101(27):10223-10228.
20. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant staphylococcus aureus. *Lancet*. 2010;375(9725):1557-1568.
21. Lekkerkerk WS, van de Sande-Bruinsma N, van der Sande MA, et al. Emergence of MRSA of unknown origin in the netherlands. *Clin Microbiol Infect*. 2012;18(7):656-661.
22. Price LB, Stegger M, Hasman H, et al. Staphylococcus aureus CC398: Host adaptation and emergence of methicillin resistance in livestock. *MBio*. 2012;3(1):10.1128/mBio.00305-11. Print 2012.
23. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005;3(12):948-958.
24. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant staphylococcus aureus in europe. *Lancet Infect Dis*. 2010;10(4):227-239.
25. Hetem DJ, Westh H, Boye K, Jarlov JO, Bonten MJ, Bootsma MC. Nosocomial transmission of community-associated methicillin-resistant staphylococcus aureus in danish hospitals. *J Antimicrob Chemother*. 2012;67(7):1775-1780.
26. Wassenberg MW, Kluytmans JA, Box AT, et al. Rapid screening of methicillin-resistant staphylococcus aureus using PCR and chromogenic agar: A prospective study to evaluate costs and effects. *Clin Microbiol Infect*. 2010;16(12):1754-1761.

CHAPTER 2: TECHNICAL APPENDIX

Statistical methods

The model of Bootsma, Wassenberg[1] predicts that the detected size of an outbreak is geometrically distributed. We denote the parameter of the geometric distribution by ξ. This means that the likelihood that a randomly chosen detected outbreak has size *i* (including the index case) is given by $\xi(1-\xi)^{i-1}$.

If there are *N* outbreaks with a total of *M* secondary cases, the likelihood of observing these outbreak sizes is given by $L = \xi^N(1-\xi)^M$. The maximum likelihood estimator, i.e. the value of the parameter ξ which makes the observations most likely, is given by $\xi_{MLE} = N/(N + M)$.

A confidence interval for ξ_{MLE} can be obtained by the profile likelihood method. This confidence interval contains all values for the parameter ξ of the geometric distribution for which the observations are still sufficiently likely. The cut-off values to determine whether the data are still sufficiently likely depends on whether we are calculating 90% confidence intervals, 95% or 99% confidence intervals and is based on the chi-square distribution.

When we know the discharge rate and the rate at which colonization is detected, we can calculate a ratio of these two. With this ratio (*r*) we can translate values for ξ into values of R_A, the per admission reproduction number by using the formula $R_A = (1- \xi)(r + \xi) / \xi$ (see Bootsma, Wassenberg et al.[1] for more details).

To check whether the model assumptions, which lead to a geometric distribution of the outbreak sizes, are in agreement with the data, we tested whether the observed outbreak sizes are indeed similar to a geometric distribution by performing the Anderson Darling goodness of fit test. This test is based on the test statistic: where $p(i)=\xi_{MLE}(1-\xi_{MLE})^{i-1}$ is the probability density function of the geometric distribution, *F(i)* is the cumulative density function corresponding to *p(i)* and is the empirical cumulative density function.

REFERENCES

1. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.

Technical appendix Table A. Mathematical parameters of index case-patients

	LA-MRSA n=40	Other MRSA n=101
Discharge rate (days)	1/13	1/10
Detection rate (days)	1/20	1/20
ξ (95% CI)	0.93 (0.83-0.98)	0.70 (0.62-0.77)
R_A (95% CI)	0.12 (0.03-0.30)	0.52 (0.38-0.69)

Note: ξ, parameter of geometric distribution.

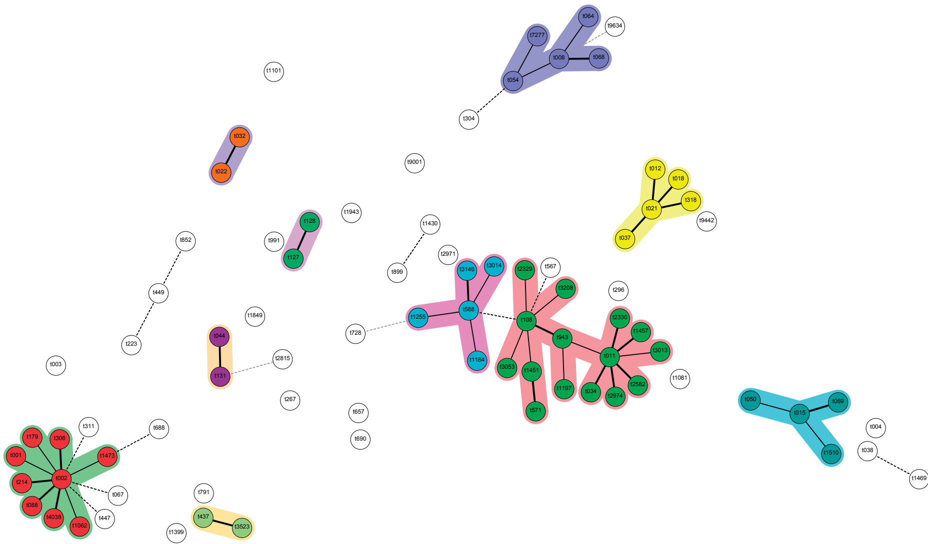


Figure 1. Technical Appendix Figure: *spa*-based minimal spanning tree
Note: Including spa types of index cases and *spa* types considered livestock associated. *spa* types with the same color are considered to be related.

three

TRANSMISSION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AFTER SHORT-TERM HEALTH CARE SETTING EXPOSURE

David J. Hetem, Martin C.J. Bootsma, Annet Troelstra and Marc J.M. Bonten

Submitted

ABSTRACT

The risk of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission after short-term exposure of MRSA carriers to a health care setting is unknown. We, therefore, quantified transmission of MRSA originating from MRSA-carriers treated without barrier precautions in outpatient clinics and during periods less than 24 hours in hospital wards. Post-contact screening was performed among patients and health care workers after identification of an index patient. During the six months study period there were 111 index cases and contact screening was performed in 1164 health-care workers and 185 patients. Transmission of MRSA after short-term exposure occurred in one of 111 episodes.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen and MRSA infections have been associated with excess morbidity, mortality and health-care expenses.[1,2] The emergence of MRSA originating in the community could jeopardize successful control of MRSA in hospitals in countries with a historically low nosocomial MRSA prevalence, such as the Netherlands and the Scandinavian countries. Community-associated MRSA (CA-MRSA) primarily causes skin and soft tissue infections, and such patients are mainly seen at outpatient clinics or emergency departments.[3] In the Netherlands, barrier precautions, including gloves and gowns, are used when known MRSA-carriers or patients with risk factors for MRSA carriage visit outpatient clinics or are admitted to hospital wards. In absence of these precautionary methods, MRSA-transmission to healthcare workers (HCW) or other patients may occur. The risk of transmission of MRSA after short-term health care contacts, including outpatient clinic visits, is unknown. Here, we quantified the risk of MRSA-transmission, in the absence of barrier precautions, in outpatient clinics and during short-term exposure in hospital wards.

METHODS

Data and definitions

All Dutch medical microbiologists and infection control practitioners were contacted and asked to prospectively collect data on hospital MRSA outbreaks and subsequent post contact screenings from September 2011-December 2011. The same data were collected retrospectively from June 2011 till September 2011. In this analysis we address the results of post-contact screening in the outpatient clinic and of patients with less than 24 hours unprotected health care exposure. Index cases were thus defined as MRSA positive patients who: 1) visited the outpatient clinic or emergency department without any precautionary measures taken or 2) who were admitted to a hospital ward without precautionary measures or isolation but in whom these measures were implemented within 24 hours after admission. Only index patients for whom post-contact screening had been performed were included as index case. From all post-contact screenings we determined numbers of screened healthcare workers (HCW) and patients and the number of secondary colonized HCW and patients. Age, gender, location of primary culture and the presence of risk factors for MRSA carriage, as described in the Dutch National MRSA guideline,[4] were collected. Only isolates from screened subjects with *spa* types related to that of the index case were considered a secondary case, all other were considered coincidental findings.

MRSA genotyping

All isolates of index and secondary cases were *spa* genotyped by the national reference laboratory (RIVM) as previously described, using the Ridom StaphType program (www.ridom.de) to allocate *spa*-types.[5] *Spa* types considered livestock associated MRSA (LA-MRSA) were: *t011, t034, t108, t567, t571, t588, t753, t753, t779, t898, t899, t943, t1184, t1197, t1254, t1255, t1451, t1456, t1457, t2123, t2287, t2329, t2330, t2383, t2582, t2748, t2971,*

three

Table 1. Characteristics of index patients with LA-MRSA and non LA-MRSA genotypes

	LA-MRSA n=31	non LA-MRSA n=80	*p*-value
Age (mean)	42	92	0.339
Male	18 (58%)	45 (56%)	0.916
Unknown risk factor	12 (39%)	60 (75%)	0.000
Location of culture			
Nose, throat, perineum	23 (74%)	23 (29%)	0.000
Skin and soft tissue	5 (16%)	50 (63%)	0.000
Respiratory tract	2 (6%)	3 (4%)	0.624
Uro-genital tract	1 (3%)	0	0.287
Deep-tissue	0	1 (1%)	1.000

Note: LA-MRSA: livestock-associated methicillin resistant Staphylococcus aureus.

t2974, t3013, t3014, t3053, t3146, or *t3208*.[6-8] Identification of *luk*-PV genes, indicative of Panton-Valentine leukocidin (PVL) was done as previously described.[9]

Statistical analysis

Nominal variable were analyzed by x^2 and by fisher exact test when appropriate. Mann-Whitney U test was used for analysis of continuous variables. A *p*-value <0.05 was considered significant. Data was analyzed using SPSS (v 15.0) for Windows.

RESULTS

During the study period 39 hospitals performed post contact screenings after identification of 111 index patients, which included 185 patients and 1164 HCW. Fifty-seven different *spa* types were identified among the 111 index patients, and one strain was non-typable. LA-MRSA t011 (n=18, 16%) was most common, followed by t019 (n=8, 7%) and t008 (n=7, 6%).

Thirty-one isolates (28%) had *spa* types considered livestock-associated with t011 being the most prevalent (n=18, 58% of all livestock genotypes). t034, t899 and t1457 were found in 6, 3 and 1 isolates respectively. (Table 2)

Genes indicative of PVL were detected in 33 (30%) isolates. In these 33 isolates, 17 different *spa* types were identified. t019 was the most common PVL-positive *spa* type (n= 8), followed by PVL positive t008. (Table 2) In 5 (5%) isolates the presence of PVL was undetermined. In none of the livestock associated genotypes, genes encoding for PVL could be detected.

Mean age of the index cases was 46 years with no significant difference between livestock and non-livestock associated genotypes (p=0.339). Index cases with a PVL positive genotype were significantly younger then the index patients with a PVL negative genotype (mean age 37 versus mean age 51, p=0.001).

Table 2. Genotyping results of index patients

Spa type	Index patients n = 111	PVL n = 33
LA-MRSA		
t011	18 (16%)	0/15
t034	6 (5%)	0/6
t899	3 (3%)	0/3
Singletons [a]	4 (4%)	0/4
non LA-MRSA		
t019	8 (7%)	8/8
t008	7 (6%)	7/7
t002	6 (5%)	2/6
t437	4 (4%)	3/3
t024	2 (2%)	1/2
t030	2 (2%)	0/2
t032	2 (2%)	0/2
t044	2 (2%)	1/2
t127	2 (2%)	0/2
t267	2 (2%)	0/2
t688	2 (2%)	0/2
t786	2 (2%)	1/2
Singletons	39 (35%) [b]	10/38 [c]

Note: PVL: Panton-Valentine leukocidin. LA-MRSA : livestock associated MRSA [a] *Spa* types: t108, t567, t1457, t2123; [b] One isolate non-typable by *spa* typing [c] PVL positive *spa* types: t005, t021, t138, t304, t318, t596, t975, t3387, t9134, t9633.

Twenty-three (74%) cultures yielding LA-MRSA were taken from nose, throat or perineum, significantly more than the 23 (29%) of the cultures yielding non LA-MRSA taken from these sites (p=0.000). In contrast, cultures taken from skin and soft tissue were predominant in the non LA-MRSA genotypes (LA-MRSA: 5 (16%), non LA-MRSA: 50 (63%); p=0.000).

Risk factors for MRSA carriage could not be retrieved in 12 (39%) of 31 index patients with LA-MRSA and in 60 (75%) of 80 index cases with a non LA-MRSA genotypes (p=<0.000). In the non LA-MRSA group, patients with PVL positive isolates were significantly less likely to have known risk factors for MRSA carriage compared to PVL negative isolates (30 (91%) vs. 29 (66%), p=0.01).

The 111 post-exposure screenings yielded 6 HCWs and 2 patients colonized with MRSA, but only two of them, both HCW, were colonized with MRSA isolates that were related to the isolate from the same corresponding index case (PVL negative, *spa* type t253). Both HCWs shared the same office and subsequent investigations demonstrated that the partner and two children of one HCW were also colonized by the same MRSA strain.

DISCUSSION

Based on the data from 39 hospitals during 6 months our findings demonstrate that transmission of MRSA after short-term exposure of MRSA-carriers to the health care setting is rare. Only in one of 111 post contact screenings two secondary cases were identified.

Thirty-eight (34%) index patients had risk factors for MRSA, with regular exposure to livestock being the most frequent risk factor. Previous studies suggested that LA-MRSA is less transmissible in the Dutch hospital setting, potentially contributing to the low transmissibility found in this study.[10,11] In Danish hospitals other community associated, but non-livestock-associated, MRSA strains were associated with lower transmissibility, which might be related to the generally younger and healthier population affected by carriage.[12] As only one transmission event was identified in this study any comparison between different genotypes is impossible.

Thirty percent of MRSA isolates were PVL positive. PVL is a pore forming toxin, associated with increased virulence and found in most community-associated MRSA (CA-MRSA). In contrast to the one major dominant clone of CA-MRSA found in the United States, i.e. USA300, the PVL positive isolates found in this study were from different clonal lineages. Seventeen different spa types were identified in 33 PVL positive isolates, with the Southwest Pacific clone (t019, PVL+) being the most common, followed by USA300 (t008, PVL+). The heterogeneity of CA-MRSA genotypes in Europe has been described before.[13] Our findings also demonstrate the heterogeneity of circulating MRSA types in the Netherlands with 57 unique *spa* types isolated from 111 index patients.

A limitation of this study is that not all participating hospitals performed screening after the identification of an index case in the outpatient settings and that screening was not performed according to a standardized protocol, potentially leading to selection bias. Performing post contact screening after MRSA-exposure in the outpatient clinic is not recommended in the National MRSA guidelines. However, it is likely that post contact screenings were performed mostly in index patients with a perceived higher chance of transmission, creating bias towards overestimation of the actual transmission risk.

CONCLUSIONS

In conclusion, transmission of MRSA after short-term exposure to a MRSA colonized or infected patient occurred in one of 111 episodes.

ACKNOWLEDGEMENTS

We would like to thank all clinical microbiologists and infection control practitioners participating in this study.

FINANCIAL SUPPORT

No financial support was received for this study.

POTENTIAL CONFLICTS OF INTEREST

All authors report no conflicts of interest relevant to this article.

REFERENCES

1. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant staphylococcus aureus and escherichia coli bacteremia: Estimating the burden of antibiotic resistance in europe. *PLoS Med*. 2011;8(10):e1001104.
2. Kaye KS, Anderson DJ, Choi Y, Link K, Thacker P, Sexton DJ. The deadly toll of invasive methicillin-resistant staphylococcus aureus infection in community hospitals. *Clin Infect Dis*. 2008;46(10):1568-1577.
3. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant staphylococcus aureus. *Lancet*. 2010;375(9725):1557-1568.
4. Infection Prevention Working Party. MRSA hospital guideline. http://www.wip.nl/UK/free_content/Richtlijnen/110530%20MRSA%20hospital%20def.pdf. Accessed 05/04, 2015.
5. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-5448.
6. Huijsdens XW, Bosch T, van Santen-Verheuvel MG, et al. Molecular characterisation of PFGE non-typable methicillin-resistant staphylococcus aureus in the netherlands, 2007. *Euro Surveill*. 2009;14(38):19335.
7. van Cleef BA, Monnet DL, Voss A, et al. Livestock-associated methicillin-resistant staphylococcus aureus in humans, europe. *Emerg Infect Dis*. 2011;17(3):502-505.
8. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. Nasal colonization of humans with methicillin-resistant staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. *PLoS One*. 2009;4(8):e6800.
9. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of panton-valentine leukocidin-producing staphylococcus aureus in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29(5):1128-1132.
10. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.
11. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus. *Emerg Infect Dis*. 2013;19(11):1797-1802.
12. Hetem DJ, Westh H, Boye K, Jarlov JO, Bonten MJ, Bootsma MC. Nosocomial transmission of community-associated methicillin-resistant staphylococcus aureus in danish hospitals. *J Antimicrob Chemother*. 2012;67(7):1775-1780.
13. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant staphylococcus aureus in europe. *Lancet Infect Dis*. 2010;10(4):227-239.

four

NOSOCOMIAL TRANSMISSION OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN DANISH HOSPITALS

David J. Hetem, Henrik Westh, Kit Boye, Jens Otto Jarløv, Marc J. M. Bonten and Martin C. J. Bootsma

Journal of Antimicrobial Chemotherapy, 2012

ABSTRACT

Objectives: The emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has changed the epidemiology of MRSA infections worldwide. In contrast to hospital-associated MRSA (HA-MRSA), CA-MRSA more frequently affects healthy individuals, both with and without recent healthcare exposure. Despite obvious epidemiological differences, it is unknown whether differences in nosocomial transmissibility exist. We have, therefore, quantified the transmissibility, expressed by the single admission reproduction number (R_A), of CA-MRSA and HA-MRSA in hospital settings in Denmark.

Methods: MRSA index cases and secondary cases were investigated in four hospitals in the Copenhagen area. Index cases were defined as non-isolated, non-screened patients with MRSA, and secondary cases were defined as persons carrying MRSA isolates—identical to that of the corresponding index—as identified through contact screening. CA-MRSA and HA-MRSA were categorized upon genotyping [CA-MRSA: t008-ST8, PVL+; t019-ST30, PVL+; t127-ST1, PVL+; t044-ST80, PVL+; and their related *spa* types; and HA-MRSA: all other (where ST stands for sequence type and PVL stands for Panton–Valentine leucocidin)]. A mathematical model was applied to determine the genotype-specific transmission rate (i.e. R_A) of CA-MRSA and HA-MRSA strains.

Results: During the 7 year study period there were 117 MRSA index cases with subsequent post-contact screening (of 1108 patients and healthcare workers), revealing 22 outbreaks with a total of 52 secondary patients. R_{A}-values were 0.07 (95% CI 0.00–0.28) and 0.65 (95% CI 0.48–0.84) for CA-MRSA and HA-MRSA, respectively.

Conclusions: In four Danish hospitals the nosocomial transmission rate of CA-MRSA was 9.3 times lower than that of HA-MRSA.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen and MRSA infections have been associated with excess morbidity and mortality.[1,2] Until 2000, MRSA was mainly confined to healthcare settings, but the emergence of community-associated MRSA (CA-MRSA) has changed the global epidemiology of MRSA infections.[3-5] While so-called hospital-associated MRSA (HA-MRSA) typically cause infections in hospitalized and frequently debilitated patients, CA-MRSA typically infect healthy individuals without previous healthcare contact. In recent years, though, CA-MRSA have become increasingly prevalent in hospital settings, especially in the USA.[6]

The rapid dissemination of MRSA strain USA300 in hospitals in the USA combined with the high attack rate during outbreaks suggests that this strain has a higher transmission capacity than HA-MRSA strains.[3] In contrast to USA300, the nosocomial transmission of livestock-associated MRSA (ST398; where ST stands for sequence type) was less likely than that of traditional HA-MRSA strains in Dutch hospitals.[7] Our understanding of the transmissibility of different MRSA genotypes, other than livestock-associated CA-MRSA in Dutch hospitals, remains largely incomplete. We, therefore, aimed to quantify the variability in the nosocomial transmissibility of CA-MRSA by calculating the single admission reproduction number (R_A) using a mathematical model based on queuing theory.

METHODS

Setting

Hvidovre Hospital is a university hospital in Copenhagen. Its Department of Clinical Microbiology serves four hospitals (Hvidovre Hospital, Amager Hospital, Bispebjerg Hospital and Frederiksberg Hospital) and general practice in Copenhagen. Herlev Hospital's Department of Clinical Microbiology serves general practice for part of the island of Amager. Patients from this part of Amager are primarily admitted to Amager Hospital.

Index patients

Clinical and demographical data from patients colonized or infected with MRSA between January 2003 and April 2010 were extracted from the laboratory information system of the Department of Clinical Microbiology of Hvidovre Hospital and Herlev Hospital, the patient administration system (Grønt System) and the Danish Civil Registration System, which tracks daily changes in vital status, including change of address, date of emigration and date of death, for the entire Danish population.[8] Age, gender, location of first isolation sample site, hospital ward of index case, total length of hospital stay and number of hospital days until isolation were extracted.

Search for secondary MRSA cases

The Danish national MRSA guidelines have been in place since 2006.[9] Between 2003 and 2006 a precursor of the national guidelines was used, which was based on the practice at Hvidovre Hospital at that time. During the study period the screening policy for MRSA did not change. Hospitalized but non-isolated patients in whom MRSA carriage (or infection) was detected (i.e. an index case) would be isolated and patients sharing the same room would be screened to determine patient-to-patient spread. In the case of documented transmission of MRSA, all other patients in the ward and all ward personnel who had had contact with the MRSA carriers would be screened as well. Patient screening included swab samples from nose, throat and perineum, and from sores, skin infections and, when present, intravenous entry or drainage sites, probes, urine samples (in the case of an indwelling catheter) and tracheal secretions (only in intubated patients). For healthcare workers, screening included swabs from nose, throat and, if present, skin infections. Secondary cases were required to have an identical MRSA genotype to that of the index case. The post-exposure screening results were retrieved and analysed for secondary MRSA cases.

MRSA isolates and typing

Samples were processed according to local protocols, which initially used blood agar and MRSA chromogenic agar and, more recently, semi-selective broth enrichment.[10] Antibiotic susceptibility testing for cefoxitin, erythromycin, clindamycin, vancomycin, gentamicin, rifampicin, moxifloxacin, fusidic acid and linezolid was done on all isolates as described elsewhere.[11] All MRSA isolates were confirmed *mecA* positive by PCR.[12] All isolates of index and secondary cases were *spa* genotyped as previously described,[13] using the Ridom StaphType program (www.ridom.de) to allocate *spa* types. The staphylococcal cassette chromosome *mec* (SCC*mec*) type and the presence of *luk*-PV genes, indicative of Panton–Valentine leucocidin (PVL), were determined by using an in-house multiplex PCR, as described elsewhere.[14,15] Multi locus sequence typing (MLST) was done as previously described[16] or the type was assigned by using the Spaserver (http://spaserver.ridom.de).

CA-MRSA were defined as belonging to the following genotypes: t008-ST8, PVL+, SCC*mec* IV (USA300); t019-ST30, PVL+, SCC*mec* IV (the south-west Pacific clone); t127-ST1, PVL+, SCC*mec* IV (USA400); or t044-ST80, PVL+, SCC*mec* IV (the European clone).[17] We considered t068, PVL+, a single-locus variant of t008, to be related to t008. All remaining genotypes were considered HA-MRSA.

Model

We use a previously described mathematical model based on queuing theory[18] to estimate the strain-specific transmission capacity, i.e. the R_{A-} value. The basic assumption of this model is that each outbreak is caused by a colonized patient who enters the hospital while all other patients are uncolonized and susceptible. We assume that when an index patient is detected as such, the contact screening will identify all colonized patients who are involved

in the outbreak and are still hospitalized. Three rates determine the spread of MRSA in the hospital setting: (i) the rate at which the MRSA strain spreads; (ii) the rate at which the MRSA-colonized patient is detected as such (i.e. microbiological cultures); and (iii) the rate at which a colonized patient can no longer be detected as such.

The model predicts that the distribution of the number of patients colonized at the time of detection of the index case is geometrically distributed. The parameter (ξ) of the geometric distribution of the detected outbreak sizes was determined by using maximum likelihood estimation. If there are N outbreaks with a total of M secondary cases, the likelihood is given by $L=\xi^N(1-\xi)^M$. The maximum likelihood estimator (MLE) is given by ξ MLE=$N/(N+M)$. The outbreak size alone is insufficient to calculate R_A. Small, detected outbreak sizes could correspond to either a low potential of transmission or to a high detection rate.

Patients with MRSA remain colonized during their hospital stay and, therefore, the infectious period ends at the time of discharge. Genotype-specific discharge rates were calculated from the admission and discharge data of index patients admitted to the four Danish hospitals during the study period. The detection rate was based on all blood, respiratory tract and wound cultures done during 2005 in the four participating hospitals. The upper detection limit consists of all these cultures divided by the total number of patient days in 2005. With the ratio between the average detection rate and genotype-specific discharge rate (r), combined with the parameter of geometric distribution ξ, we can calculate R_A [$R_A=(1-\xi)(r+\xi)/\xi$].

Statistical analysis

Significance was assessed using two-sided tests for all variables, applying a cut-off value of $P=0.05$. CIs were calculated using the profile likelihood method. The Anderson–Darling goodness-of-fit test was used to assess whether the outbreak sizes were geometrically distributed.

RESULTS

During the 7 year period there were 124 MRSA index patients with subsequent post-contact screening among patients and healthcare workers ($n=1108$) in the four participating hospitals.

Seven index patients were detected through screening cultures: three were screened on hospital admission but not isolated and four patients were screened after a period in which no barrier precautions were taken during hospital admission. These 7 patients were excluded, leaving 117 index patients for further analysis.

Among the 117 index patients, 24 different *spa* types were identified (see Table 1), with t024 ST8 IV (50%) being most frequent, followed by t008 ST8 IV (7%), t019 ST30 IV (5%), t002 ST5 II (4%) and t4866 (4%). SCC*mec* typing revealed 98 isolates with SCC*mec* type IV

Table 1. Genotypes of MRSA from index and secondary cases

spa type	MLST	PVL	Index cases *n*= 117	Number of outbreaks *n*= 22	Secondary patients *n*= 52
CA-MRSA					
t019[a]	ST30	6/6 (100%)	6 (5%)	0	0
t008[b]	ST8	5/5 (100%)	5 (4%)	1 (4%)	1 (2%)
t044[c]	ST80	3/3 (100%)	3 (3%)	0	0
t068	ST8	1/1 (100%)	1 (1%)	0	0
HA-MRSA					
t024	ST8	0/57	58 (50%)	11 (50%)	22 (42%)
t002	ST5	1/4 (25%)	5 (4%)	2 (9%)	5 (10%)
t4866	NT	0/4	5 (4%)	0	0
t003	ST5	0/4	4 (3%)	2 (9%)	5 (10%)
t032	ST22	0/4	4 (3%)	0	0
t008	ST8	0/3	3 (3%)	0	0
t430	ST8	0/3	3 (3%)	1 (4%)	1 (2%)
t5147	ST22	0/3	3 (3%)	0	0
t223	ST22	0/2	2 (2%)	1 (4%)	5 (10%)
t015	ST45	0/2	2 (2%)	2 (9%)	10 (19%)
t379	ST22	0/2	2 (2%)	1 (4%)	1 (2%)
t064	ST8	0/2	2 (2%)	0	0
singletons[d]		0/9	9 (8%)	1 (4%)[e]	2 (4%)

Note: NT, non-typeable. [a]South-west Pacific clone. [b]USA300. [c]European clone. [d]HA: t005, t021, t037, t062, t421, t844, t127, t122 and t267. [e]Outbreak was caused by PVL-negative t127.

or V (84%). HA-MRSA SCC*mec* types I, II and III were detected in one, four and two index patients, respectively (6% combined). In 12 strains (10%), SCC*mec* was non-typeable. The *luk*-PV gene, indicative of PVL, was detected in 16 (14%) of the 117 isolates investigated. Fifteen of these isolates (94%) belonged to CA-MRSA genotypes. The PVL-positive *spa* types were t002, t008, t019, t044 and t068 (Table 1).

The CA-MRSA isolates were more frequently susceptible to erythromycin (P=0.004) and clindamycin (P=0.001) (Table 2). Twenty percent of the CA-MRSA genotypes and 38% of the HA-MRSA genotypes were resistant to more than two non-β-lactam antibiotics (P=0.169).

Fifteen index patients (13%) had community-associated genotypes, with t019 ST30 IV being the most common (n=6). Index patients with CA-MRSA were younger than index patients with HA-MRSA (mean age 49 versus 72 years, P=0.006). Furthermore, the length of hospital stay and the number of non-isolated days were shorter in index cases with CA-MRSA (Table 2). Eighty percent of the CA-MRSA strains were isolated from skin and soft tissue infections (SSTIs), as were 44% of the HA-MRSA isolates (Table 2).

Table 2. Summary of results and characteristics of index cases

	CA-MRSA n=15	HA-MRSA n=102	*P* value
Discharge rate (days)	1/3	1/21	
Detection rate (days)	1/33.6	1/33.6	
ξ (95% CI)	0.94 (0.75–1.00)	0.66 (0.59–0.74)	
R_A (95% CI)	0.07 (0.00–0.28)	0.65 (0.48–0.84)	
Age (years, mean)	49	72	0.006
Length of stay (days, median)	3	21	0.007
Non-isolated days (days, median)	3	9	0.017
Culture location			
SSTI	12	45	0.005
urinary tract	1	21	0.198
respiratory tract	1	12	0.557
deep tissue	1	14	0.445
blood	0	10	0.205
Antibiotic susceptibility			
cefoxitin	0	0	
erythromycin	9 (60%)	24 (24%)	0.004
clindamycin	9 (75%)	25 (26%)	0.001
moxifloxacin	10 (71%)	54 (54%)	0.218
gentamicin	15 (100%)	95 (93%)	0.295
rifampicin	13 (100%)	86 (92%)	0.274
fusidic acid	12 (86%)	96 (94%)	0.245
non-multiresistant[a]	12 (80%)	63 (62%)	0.169

Note: ξ, parameter of geometric distribution. Isolates tested: erythromycin, 114/117 (97%); clindamycin, 107/117 (91%); moxifloxacin, 114/117 (97%); gentamicin 117/117 (100%); rifampicin, 107/117 (91%); and fusidic acid, 116/117 (99%). [a]Non-multiresistant: resistant to two or less non-β-lactam antibiotics.

MRSA carriage was detected in 55 of 1108 (5%) persons screened during contact investigations. Three persons (two patients and one healthcare worker) had MRSA strains with different *spa* types than detected in the index patients and, thus, were not considered secondary cases. In 22 index patients, secondary cases were identified, with outbreak sizes ranging from 1 to 9 patients (excluding the index case, see Figure 1). The majority of outbreaks (12 of 22, 55%) consisted of one secondary case (Figure 1).

In 2005, there were 116836 admissions to the four hospitals. On average, each hospital had 737029 patient days per year, with an average stay of 6.3 days per admission. Yet, for index patients the average length of hospital stay was 19 days, with marked differences between hospital-associated and community-associated genotypes (21 versus 3 days). In the four

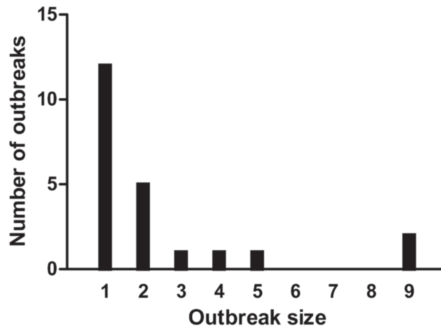


Figure 1. Histogram of the outbreak sizes (number of secondary cases excluding the index case).

hospitals, 16167 blood cultures, 3155 wound cultures and 8011 respiratory tract cultures were performed in 2005, yielding 21234 relevant cultures (maximum of one culture per patient per day). Assuming that every culture would detect MRSA in a colonized or infected patient, the upper limit for the MRSA detection rate is 21234/737029 per patient day. Therefore, an index case will be detected as such every 33.6 days of colonization. The genotype-specific ratio between discharge and detection is 1.77, 11.19 and 1.60 for all genotypes, community-associated genotypes and hospital-associated genotypes, respectively.

The parameter for the geometric distribution, ξ, was 0.69 (95% CI 0.62–0.76), 0.94 (95% CI 0.75–1.00) and 0.66 (95% CI 0.59–0.74) for all genotypes, CA-MRSA and HA-MRSA, respectively. There was no reason to reject the hypothesis of a geometrically distributed outbreak size for CA-MRSA (P=1). However, for hospital-associated strains the hypothesis of a geometrically distributed outbreak size was rejected (P=0.009).

Based on the genotype-specific discharge/detection ratios, R_A values (i.e. the single admission transmissibility rates) were 0.07 (95% CI 0.00–0.28) and 0.65 (95% CI 0.48–0.84) for CA-MRSA and HA-MRSA, respectively.

DISCUSSION

Using the observational data of patients colonized with different genotypes of MRSA in four Danish hospitals, the results of subsequent contact investigations to determine MRSA transmission and a mathematical model, we conclude that CA-MRSA is 9.3 times less transmissible than HA-MRSA in Danish hospital settings. The R_A values found are probably an overestimation of the actual risk of transmission, considering the fact that all cultures would not necessarily lead to the detection of an index case.

In this study we defined CA-MRSA based on combinations of genotypes, which are all known to cause infection in the community. It is becoming increasingly difficult to categorize CA-MRSA and HA-MRSA upon epidemiological criteria, such as duration of healthcare exposure. Since typical CA-MRSA strains may cause nosocomial infections[19,20] and HA-MRSA

genotypes may circulate in the community, such a definition will lead to misclassification. In fact, CA-MRSA isolates have already become the dominant isolates causing hospital infections in some regions.[6] The differentiation of MRSA isolates by one or multiple genotypic and phenotypic characteristics is, therefore, to be preferred. PFGE patterns, *spa* type, MLST, SCC*mec* type, presence of PVL and antibiotic susceptibility profiles have been used, alone or in combination, for this purpose. However, some features traditionally considered to be specific for CA-MRSA, e.g. SCC*mec* type IV, can also be found in successful hospital-associated isolates.[21,22] Here, we use a combination of *spa* type and PVL to define community-associated genotypes. Isolates originating from the community could have been regarded as HA-MRSA; however, this would only dilute the increased transmissibility of HA-MRSA. Of the *spa* types considered to be community associated, only t008 had both PVL-positive and PVL-negative isolates, and only PVL-positive t008 isolates were considered community associated. Including the PVL-negative t008 isolates as community associated would further increase the differences in the R_A values between HA-MRSA and CA-MRSA. The low number of index patients and, in particular, the low number of index patients with CA-MRSA isolates are a potential limitation to this study.

Our findings from the present study are comparable to the results of a recent study in which the transmissibility of livestock-associated MRSA was—using the same methods—compared with HA-MRSA strains in Dutch hospitals.[23] In both studies, the R_A of HA-MRSA (and its 95% CI) was <1, implying that a single admission of a patient colonized with MRSA is unlikely to initiate an epidemic, which confirms previous estimates of the R_A of HA-MRSA.[24,25] The comparability of the R_A ratios of community genotypes found in Denmark and livestock genotypes found in the Netherlands is hard to determine. Probable dissimilarities in healthcare systems, nursing and ward protocols between countries are important factors that influence R_A. As livestock-associated MRSA is rarely encountered in the Copenhagen area, we could not estimate the R_A values of these strains in Danish hospitals. However, there is considerable genetic diversity of MRSA strains, including CA-MRSA, in the Copenhagen area, with the PVL-negative t024 ST8 IV strain being most prevalent.[26] This strain was responsible for multiple outbreaks in nursing homes during the study period and was introduced into the hospitals via the admission of long-term residents of these nursing homes. Since 2005, residents admitted from nursing homes with documented MRSA outbreaks have been considered at risk for MRSA and, therefore, are screened and subsequently isolated at hospital admission.

The recently reported successful emergence of CA-MRSA, USA300 in particular, in hospitals in the USA seems contradictory to the low transmissibility of CA-MRSA in Danish hospitals. Yet, the high transmissibility of USA300 in the community, especially among people with direct skin-to-skin contact,[27] may well have increased the introduction of CA-MRSA into hospitals. One could also speculate that an increase in MRSA colonization in healthcare workers could subsequently lead to an increase in transmission in hospitals. Importantly, frequent introductions of a pathogen with low transmissibility may still lead to

patient-to-patient transmission. Furthermore, differences between US and Danish hospitals in general infection control practices and other variables influencing transmission from non-isolated patients (such as contact rates, and consequently staffing levels, beds per room, room sizes and patient turn-around time) will also influence the transmissibility of CA-MRSA. Finally, the characteristics of patients colonized or infected with CA-MRSA could be different between US and Danish hospitals, and this would also influence R_A values.

four

For this model it is essential that outbreaks are rare and that rigorous screening occurs after the identification of an index case. If multiple outbreaks of the same genotype occur on the same ward the R_A will be an overestimation, given the fact that all cases found after secondary screening will be attributed to the first index case. The 117 index cases found in four hospitals during 88 months of MRSA policy, is in line with this assumption. Furthermore, we have made some critical assumptions about heterogeneity. First, no distinction was made between healthcare workers and patients. Second, we assumed all carriers were equally infective and that all susceptible persons were equally at risk of becoming subsequently colonized. The consequences of these assumptions have been discussed in more detail elsewhere.[23]

The difference in the R_A values between CA-MRSA and HA-MRSA isolates could result from host factors, bacterial characteristics or a combination of both. The differences in patient age and length of hospital stay suggest that patients infected with or carrying CA-MRSA differ from those with HA-MRSA: index patients with CA-MRSA are younger and are discharged faster, which suggests that they are in a better health condition and is in accord with the general conception that CA-MRSA more frequently affects healthy individuals without previous healthcare exposure. Furthermore, more CA-MRSA infections were caused by SSTIs, which potentially accelerated the detection and implementation of isolation precautions. It is possible that these patients are less likely to spread MRSA and that those who share their hospital room are less susceptible to acquisition, due to differences in the severity of illness.

FUNDING

This study was supported by a grant from the European Community: CONCORD FP7; EC222718.

TRANSPARENCY DECLARATIONS

None to declare.

ACKNOWLEDGEMENTS

We would like to thank Heidi Meineche and Kirsten Kristoffersen for helping with the data collection.

REFERENCES

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant staphylococcus aureus as a public-health threat. *Lancet*. 2006;368(9538):874-885.
2. Styers D, Sheehan DJ, Hogan P, Sahm DF. Laboratory-based surveillance of current antimicrobial resistance patterns and trends among staphylococcus aureus: 2005 status in the united states. *Ann Clin Microbiol Antimicrob*. 2006;5:2.
3. Crum NF, Lee RU, Thornton SA, et al. Fifteen-year study of the changing epidemiology of methicillin-resistant staphylococcus aureus. *Am J Med*. 2006;119(11):943-951.
4. Liu C, Graber CJ, Karr M, et al. A population-based study of the incidence and molecular epidemiology of methicillin-resistant staphylococcus aureus disease in san francisco, 2004-2005. *Clin Infect Dis*. 2008;46(11):1637-1646.
5. van Loo I, Huijsdens X, Tiemersma E, et al. Emergence of methicillin-resistant staphylococcus aureus of animal origin in humans. *Emerg Infect Dis*. 2007;13(12):1834-1839.
6. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis*. 2008;46(6):787-794.
7. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus (ST398) in dutch hospitals. *Clin Microbiol Infect*. 2011;17(2):316-319.
8. Pedersen CB, Gotzsche H, Moller JO, Mortensen PB. The danish civil registration system. A cohort of eight million persons. *Dan Med Bull*. 2006;53(4):441-449.
9. Sundhedsstyrelsen. Vejledning om forebyggelse af spredning af MRSA. http://www.sst.dk/Sundhed%20og%20forebyggelse/Smitsomme%20sygdomme/MRSA/MRSA%20vejledning.aspx. Published 2006. Accessed 11/8, 2011.
10. Bocher S, Middendorf B, Westh H, et al. Semi-selective broth improves screening for methicillin-resistant staphylococcus aureus. *J Antimicrob Chemother*. 2010;65(4):717-720.
11. Bartels MD, Boye K, Rhod Larsen A, Skov R, Westh H. Rapid increase of genetically diverse methicillin-resistant staphylococcus aureus, copenhagen, denmark. *Emerg Infect Dis*. 2007;13(10):1533-1540.
12. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant staphylococcus aureus. *Antimicrob Agents Chemother*. 2002;46(7):2155-2161.
13. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-5448.
14. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant staphylococcus aureus: 'SCCmec IV multiplex'. *J Antimicrob Chemother*. 2007;60(1):42-48.
15. Boye K, Bartels MD, Andersen IS, Moller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant staphylococcus aureus SCCmec types I-V. *Clin Microbiol Infect*. 2007;13(7):725-727.
16. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of staphylococcus aureus. *J Clin Microbiol*. 2000;38(3):1008-1015.
17. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant staphylococcus aureus in europe. *Lancet Infect Dis*. 2010;10(4):227-239.

18. Trapman P, Bootsma MC. A useful relationship between epidemiology and queueing theory: The distribution of the number of infectives at the moment of the first detection. *Math Biosci*. 2009;219(1):15-22.
19. David MZ, Siegel JD, Chambers HF, Daum RS. Determining whether methicillin-resistant staphylococcus aureus is associated with health care. *JAMA*. 2008;299(5):519; author reply 519-20.
20. David MZ, Glikman D, Crawford SE, et al. What is community-associated methicillin-resistant staphylococcus aureus? *J Infect Dis*. 2008;197(9):1235-1243.
21. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant staphylococcus aureus (MRSA). *Proc Natl Acad Sci U S A*. 2002;99(11):7687-7692.
22. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying panton-valentine leucocidin genes in england and wales: Frequency, characterization, and association with clinical disease. *J Clin Microbiol*. 2005;43(5):2384-2390.
23. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.
24. Kajita E, Okano JT, Bodine EN, Layne SP, Blower S. Modelling an outbreak of an emerging pathogen. *Nat Rev Microbiol*. 2007;5(9):700-709.
25. Cooper BS, Medley GF, Stone SP, et al. Methicillin-resistant staphylococcus aureus in hospitals and the community: Stealth dynamics and control catastrophes. *Proc Natl Acad Sci U S A*. 2004;101(27):10223-10228.
26. Bartels MD, Kristoffersen K, Boye K, Westh H. Rise and subsequent decline of community-associated methicillin resistant staphylococcus aureus ST30-IVc in copenhagen, denmark through an effective search and destroy policy. *Clin Microbiol Infect*. 2010;16(1):78-83.
27. Hota B, Ellenbogen C, Hayden MK, Aroutcheva A, Rice TW, Weinstein RA. Community-associated methicillin-resistant staphylococcus aureus skin and soft tissue infections at a public hospital: Do public housing and incarceration amplify transmission? *Arch Intern Med*. 2007;167(10):1026-1033.

five

MOLECULAR EPIDEMIOLOGY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN THIRTEEN EUROPEAN INTENSIVE CARE UNITS

David J. Hetem, Lennie P.G. Derde, Joanna Empel, Aneta Mroczkowska, Monika Orczykowska-Kotyna, Aleksandra Kozińska, Waleria Hryniewicz, Herman Goossens and Marc J.M. Bonten on behalf of the MOSAR WP3 study group

Submitted

ABSTRACT

Background: The European epidemiology of MRSA is changing with the emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). In this study we investigated the molecular epidemiology of MRSA during two-years in 13 ICUs in France, Greece, Italy, Latvia, Luxemburg, Portugal, Slovenia and Spain.

Methods: Surveillance cultures for MRSA from nose and wounds were obtained on admission and twice weekly of all patients admitted to the Intensive Care Unit for at least three days. The first MRSA isolate per patient was genotyped in a central laboratory by MLST, *spa*-typing, *agr*-typing, and SCC*mec*-(sub)typing. Risk factors for patients with an unknown history of MRSA colonization were identified.

Results: In all, 14,390 patients were screened of whom 8,519 stayed in an ICU for $\geq$ 3 days. Overall MRSA admission prevalence was 3.9% and ranged from 1.0% to 6.4% for individual ICUs. Overall MRSA acquisition rate was 2.5/1,000 patient days at risk, and ranged from 0.2 to 8/1,000 patient days at risk per ICU. In total, 557 putative MRSA isolates were submitted to the central laboratory for typing, of which 511 (92%) were confirmed as MRSA. Each country had a distinct epidemiology, with ST8-IVc (UK-EMRSA -2/-6, USA500) being most prevalent, especially in France and Spain, and detected in ICUs in five of eight countries. Seventeen (4%) and three isolates (<1%) were categorized as CA-MRSA and LA-MRSA, respectively. Risk factors for MRSA carriage on ICU admission were age above 70 and hospitalization within one year prior to ICU admission.

Conclusions: The molecular epidemiology of MRSA in 13 European ICUs in eight countries was homogeneous within, but heterogeneous between countries. CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were detected sporadically.

BACKGROUND

MRSA can colonize and infect hospitalized and non-hospitalized humans. It is the leading nosocomial pathogen, and hospital-acquired MRSA (HA-MRSA) infections are associated with high morbidity and mortality, and increased healthcare spending.[1,2]

The global epidemiology of MRSA has changed with the emergence of community-associated and livestock-associated MRSA. Ten years ago MRSA was regarded as a sole nosocomial pathogen, mainly affecting patients with healthcare exposure, invasive medical devices, high age and undergoing surgical procedures. Since then we have witnessed a rapid increase of MRSA infections occurring in previously healthy non-hospitalized persons, so-called community-associated MRSA (CA-MRSA).[3] The risk factors for developing CA-MRSA infections differ from the traditional healthcare related risk factors for HA-MRSA infections and include crowding, lack of cleanliness and participation in contact sports. In the United States, CA-MRSA (predominantly USA300) became the most important pathogen for community-acquired skin and soft tissue infections and it has replaced traditional healthcare associated strains in being the most common strain causing nosocomial MRSA bacteremia.[4] In Europe most nosocomial MRSA infections are still caused by HA-MRSA genotypes.[5,6] Yet, in Europe animals in the agricultural industry have become a large reservoir of livestock-associated MRSA (LA-MRSA, predominantly ST398), currently accounting for 40% of all MRSA colonization and infections in the Netherlands, a country with traditionally low prevalence of MRSA.[7]

It is unknown to what extent the global changes in MRSA epidemiology affect ICU populations in Europe. We, therefore, determined prevalence, acquisition rates and molecular epidemiology of MRSA in 13 ICUs in eight European countries that participated in a prospective trial to control transmission of antibiotic resistance in ICUs.[8]

METHODS

We performed a post-hoc analysis from the ICU trial within the Mastering Hospital Antimicrobial Resistance in Europe (MOSAR) project (http://www.clinicaltrials.gov number NCT00976638), that evaluated different interventions to reduce transmission of antibiotic-resistant nosocomial pathogens including MRSA, vancomycin-resistant enterococci (VRE) and highly-resistant Enterobacteriaceae (HRE) in ICUs. The study was conducted in 13 ICUs from eight European countries: France (three ICUs); Greece (two ICUs); Portugal (two ICUs); Slovenia (two ICUs); Italy (one ICU); Latvia (one ICU); Luxembourg (one ICU) and Spain (one ICU). Written approval of the study protocol was obtained from each institution's review board or national ethics committee when required.

Design and data collection

Data were obtained during the clinical trial between May 2008, and April 2011, that comprised a six months baseline period (phase I), a six months period with implementation of a hand hygiene improvement program (largely based on the WHO "five moments" program), and feedback of compliance to personnel, as well as universal chlorhexidine body washings (phase II), followed by a 12-15 month cluster-randomized intervention phase (phase III) as described in detail elsewhere.[8]

In phase III six ICUs were randomized to chromogenic agar-based screening for MRSA and seven were randomized to PCR-based screening for MRSA, both including feedback of screening results (from either cultures on chromogenic media or molecular tests) to personnel, and the use of contact precautions for identified carriers.

five

Nasal and wound swabs were obtained from all patients admitted to an ICU for three days or longer and within 48 hours of ICU admission and twice weekly thereafter. Culture frequency was reduced to once weekly after 21 days of ICU admission.

MRSA colonization was considered ICU acquired if detected on or after the third day of ICU admission, in the absence of colonization on admission. The MRSA admission prevalence and MRSA acquisition risk per 1000 patient days at risk were calculated for all ICUs and countries. Risk factor analysis for MRSA colonization on ICU admission was performed for patients with no known history of MRSA carriage or infection. Aggregated data of thirteen ICUs was used for the identification of risk factors.

Microbiology

Nasal and wound swabs were tested in local laboratories for the presence of MRSA by chromogenic agar for MRSA detection (BBL CHROMagar MRSA II, Becton, Dickinson and Company, Franklin Lakes, NJUSA) in all study phases, including both arms of phase III, throughout the trial. ICUs randomized to rapid MRSA detection by PCR additionally used a GeneXpert real-time PCR system in phase III (Cepheid, Sunnyvale, CA, USA) on the admission swabs. Surveillance cultures were stored in cryopreservative fluid for a minimum of two months before analysis, to prevent feedback of results to clinicians. Only admission cultures in phase III were directly communicated to the wards and treating physician. All participating laboratories were required to perform proficiency panels for MRSA detection.[9]

The first MRSA isolate of each patient was sent to a central laboratory (the National Medicines Institute, Warsaw, Poland) for confirmation on both the phenotypic and genotypic level. Here, all isolates were re-identified using routine microbiological methods, including slide agglutination (Prolex, Staph Xtra Latex Kit; PRO-LAB Diagnostics, Richmond Hill, ON, Canada) in combination with coagulase. The presence of *mec*A and *lukS/lukF* genes, indicative of presence of Panton-Valentine leucocidin, were determined by PCR as described elsewhere.[10,11] *mec*A-negative isolates were additionally screened for *mec*C gene as described in Cuny et al.[12] All MRSA isolates were characterised by *spa*-typing, using the Ridom's StaphsType program to allocate *spa* types.[13] Accessory gene regulator (*agr*)

allotypes were determined according to Gilot et al.[14] Staphylococcal cassette chromosome *mec* (SCC*mec*) typing and sub-typing was performed as previously described.[15-18] SCC*mec* was classified according to guidelines proposed by the International Working Group on the Classification of SCC elements (IWGSCC, 2009).[19] Each isolate was assigned to MRSA clone (or related) as previously described.[20] Multi-locus sequence typing (MLST) according to Enright et al. was performed on at least one isolate from each *spa* type detected in predicted MRSA clones from each ICU.[21] Sequence types (ST) were assigned through the *S. aureus* MLST database (http://saureus.mlst.net). In case of USA300 clone (n=1) presence of the arginine catabolic mobile element (ACME) was confirmed by duplex PCR with primers AIPS.29 and AIPS.28 (locus *arc*) and AIPS.45 and AIPS.46 (locus *opp3*).[22]

Two clones: ST130-XI and ST398-IVa were considered LA-MRSA.[12,23] We used a molecular genotypic definition for the identification of CA-MRSA, and we considered the following clones to be CA-MRSA: ST1-IVa (USA400); ST8-IVa, *pvl* and ACME positive (USA300); ST30-IVc (the Southwest Pacific related clone); ST80-IVc, *pvl* positive (the European clone); ST88-IVa and ST152-V, *pvl* positive (Balkan clone).[3,24] Simpson's index of diversity was calculated as previously described using Ridom's EpiCompare software (version 1.0).[25,26]

Statistical analysis

Univariate analysis was performed using the chi-square or student t-test where appropriate. Risk factors with a *p*-value < 0.1 in the univariate analysis were subsequently analyzed by backward stepwise regression analysis to calculate odds ratios (OR) and 95% confidence intervals (95% CI). Significance was assessed two sided for all variables, applying a cutoff value of $p< 0.05$. Data analysis was performed with SPSS v20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Admission prevalence and acquisition

During the study period of 24-27 months 14,390 patients were screened upon admission to the ICU, of whom 8,976 were admitted for at least three days, of which from 8,519 of 8,976 (95%) patients at least one nasal was taken during ICU admission and these patients were therefore subsequently analyzed. From 931 (10%) of 8976 patients additional wound swabs were obtained. A total of 631 MRSA colonized patients were detected in the local laboratories, of which 335 (53%) of these patients were colonized on admission (Table 1). MRSA prevalence on ICU admission was 3.9% (335 of 8,519 patients) across all ICUs during the study period, and was 4.3%, 4.2% and 3.7% during phase I, phase II and phase III, respectively. The highest admission prevalence (7.0%) was observed in one of the Greek ICUs, followed by a Portuguese (6.4%) and French ICU (5.4%). Admission prevalence in other ICUs ranged from 1.0% - 5.0% (Table 1). MRSA colonization on admission was identified in 2.9%, 4.4% and 8.0% of patients admitted to the ICU directly from home, from a hospital ward or long-term care facility respectively.

Table 1. MRSA admission prevalence and MRSA acquisition rates across countries and ICUs.

Country / ICU	Patients screened n=8519	MRSA colonization on admission n=335	MRSA colonization through acquisition n=296	Acquisition of MRSA / 1000 pt. days at risk
France				
ICU 1	1419	77 (5.4%)	22 (1.6%)	1.4
ICU 2	666	33 (5.0%)	23 (3.5%)	2.3
ICU 3	502	14 (2.8%)	5 (1.0%)	0.8
Latvia	921	40 (4.3%)	90 (9.8%)	8.0
Portugal				
ICU 1	408	26 (6.4%)	28 (6.9%)	6.8
ICU 2	615	19 (3.1%)	34 (5.5%)	4.5
Italy	534	20 (3.7%)	13 (2.4%)	1.9
Greece				
ICU 1	704	49 (7.0%)	52 (7.4%)	3.8
ICU 2	268	6 (2.2%)	1 (0.4%)	0.2
Slovenia				
ICU 1	422	17 (4.0%)	12 (2.8%)	1.7
ICU 2	505	5 (1.0%)	4 (0.8%)	0.6
Spain	638	17 (2.7%)	3 (0.5%)	0.5
Luxembourg	917	12 (1.3%)	9 (1.0%)	0.7
Total	8519	335	296	2.5

Notes: MRSA: methicillin-resistant *Staphylococcus aureus*, ICU: intensive-care unit, pt: patient.

The overall MRSA-acquisition rate was 2.5 per 1,000 patient days at risk during the two year study period and 3.5, 3.1 and 2.0 per 1,000 patient days at risk during phase I, II and III, respectively. Acquisition rates of MRSA for individual ICUs ranged from 0.2 – 8.0 per 1,000 patient days, being the highest in Latvia and the lowest in one ICU from Greece (Table 1).

Fifty patients (0.6%) had MRSA bacteremia: 17 on admission, two before admission and 31 acquired during ICU stay. Twenty-three (78%) of 31 patients with ICU acquired MRSA bacteremia were colonized on admission (n=7) or acquired MRSA colonization during ICU stay before bacteremia (n=16).

Risk factors

Risk factors for MRSA colonization on admission were analyzed for 8196 patients with no known history of MRSA carriage or infection. Univariate analysis on the aggregated data of all thirteen ICUs found age > 70 years, haemodialysis, chronic hepatic failure, recent hospitalization (<1 year), recent surgery (<1 year) and admission to the ICU from a long-term

care facility being significant risk factors (Table 2). Subsequent multivariate analysis identified an age above 70 and recent hospitalization as risk factors for colonization on ICU admission (Table 3).

Table 2. Univariate analysis of risk factors for previously unknown MRSA colonization on ICU admission from all ICUs combined.

	MRSA colonization upon ICU admission			
	MRSA colonized n=223	No MRSA colonization n=7973	*p*-value	OR (95%CI)
Female gender	82 (37%)	3176 (40%)	0.357	
Age, median years	65	62	0.005	
Age >70 years	98 (44%)	2960 (37%)	0.038	1.33 (1.02-1.74)
Comorbidities				
Haemodialysis	10 (5%)	197 (3%)	0.059	1.85 (0.97-3.55)
Peritoneal dialysis	0	43 (0.5%)	0.272	
Solid tumor	29 (13%)	1109 (14%)	0.700	
Haematologic malignancy	7 (3%)	333 (4%)	0.443	
Stem cell or bone marrow transplant	2 (1%)	60 (1%)	0.806	
Solid organ transplant	4 (2%)	139 (2%)	0.955	
Chronic hepatic failure	17 (8%)	345 (4%)	0.018	1.83 (1.10-3.03)
HIV	3 (1%)	120 (2%)	0.847	
Recent hospitalization (<1 year)[a]	125 (57%)	3858 (49%)	0.016	1.34 (1.01-1.83)
Recent surgery (<1 year)[a]	55 (25%)	1539 (19%)	0.038	1.39 (1.02-1.89)
Emergency surgery prior to admission	32 (15%)	1293 (16%)	0.492	
Location prior to ICU admission				
Home or private residence	74 (33%)	3030 (38%)	1.000[b]	
Non-ICU ward, non-surgical	70 (31%)	2513 (32%)	0.436	
Non-ICU ward, surgical	38 (17%)	1166 (15%)	0.153	
Other ICU	24 (11%)	858 (11%)	0.568	
Long term care facility	8 (4%)	120 (2%)	0.006	2.73 (1.29-5.79)

Note: MRSA: methicillin-resistant *Staphylococcus aureus*, ICU: intensive-care unit, HIV: human immuno-deficiency virus, VRE: vancomycin resistant enterococci, ESBL: extended spectrum beta-lactamases. [a] Hospitalization > 24 hours [b] Reference category.

Table 3. Multivariate analysis of risk factors for previously unknown MRSA carriage on ICU admission on aggregated data.

Risk factor	OR	95% CI	*p*-value
Age >70 years	1.57	1.01-2.45	0.046
Recent hospitalization (<1 year) [a]	1.85	1.19-2.89	0.007
Location prior to ICU: long-term care facility	2.02	0.94-4.37	0.074

Note: MRSA: methicillin-resistant *Staphylococcus aureus*, ICU: intensive-care unit, OR: Odds ratio, CI: confidence interval. [a] Hospitalization > 24 hours

Genotyping

In all, 631 patients were colonized with MRSA according to local test results, and 557 putative isolates (88%) were submitted to the central laboratory, of which 511 (92%) were confirmed as MRSA. Thirty-four (6%) were methicillin-susceptible *S. aureus* (MSSA), seven (1%) were CoNS and five (<1%) appeared a non-staphylococcal species. One patient had two different MRSA genotypes, isolated during different admissions. From eleven patients identical MRSA isolates were submitted from different ICU admissions, which were excluded for the subsequent analysis.

Of the 500 MRSA isolates 480 (96%) were categorized as HA-MRSA, 17 (3%) as CA-MRSA and three (<1%) as LA-MRSA. The Brazilian/Hungarian clone ST368-III (n=99)) was most prevalent and was solely found in the Latvian ICU (99 of 105 (94%) isolates) (Table 4). ST8-IVc (UK-EMRSA -2/-6, USA500) was the most prevalent clone in ICUs in France and Spain (France: 85/157 (54%), Spain 8/18 (44%)), and this type was detected in seven ICUs in five of eight countries. ST22-IVh (UK EMRSA-15) was detected in seven ICUs in five countries. All participating ICUs had a distinct molecular epidemiology and there was little homogeneity in isolated genotypes between countries. Only ICUs from two sets of countries (France and Spain; Italy and Luxembourg) shared dominant clones. A high level of homogeneity in sequence types existed in ICUs within the same country (Table 4). The three most common found *spa* types across countries and ICUs can be found in Table 4.

In eBURST analysis MRSA isolates were grouped in eleven clonal complexes, with 253 isolates (51%) belonging to CC8/239. Other prevalent clonal complexes were CC5 (n=129, 26%) and CC22 (n=79, 16%). All clonal complexes identified in each country during the study period can be found in Figure 1.

SCC*mec*-typing revealed 211 (42%) SCC*mec* type IV, 141 (28%) SCC*mec* type III, 69 (14%) SCC*mec* type II, 40 (8%) SCC*mec* type I, 34 (7%) SCC*mec* type VI, four (1%) SCC*mec* type V and one SCC*mec* type XI (Table 5).

In seven (1.4%) isolates, obtained in five countries, *pvl* was detected, of which six were detected in CA-MRSA clones (Table 6). Typing of *agr* revealed type 1 in 334 (67%) isolates.

Table 4. Molecular epidemiology of MRSA strains across countries and ICUs.

Country / ICU	Typed MRSA isolates n=500	Number of different clones	Most common isolated clones (n, %)			Most common isolated *spa* types (n, %)			Index of diversity (95% CI)
			1st (%)	2nd (%)	3rd (%)	1st (%)	2nd (%)	3rd (%)	
France									
ICU 1	91	13	ST8-IVc 52 (57%)	ST5-VI 17 (19%)	ST247-I 6 (7%)	t008 (37, 41%)	t777 (18, 20%)	t052 (6, 7%)	0.64 (0.54-0.74)
ICU 2	50	6	ST8-IVc 27 (54%)	ST5-VI 13 (26%)	ST247-I 5 (10%)	t008 (21, 42%)	t777 (13, 26%)	t024 (5, 10%)	0.64 (0.53-0.75)
ICU 3	16	8	ST8-IVc 6 (38%)	ST5-VI 3 (19%)	ST247-I 2 (13%)	t008 (4, 25%)	t777 (3, 19%)	t002 (2, 13%)	0.84 (0.71-0.98)
Latvia	105	4	ST368-III 99 (94%)	ST22-IVa 4 (4%)	ST5-IVa 1 (1%)	t425 (78, 74%)	t3563 (19, 18%)	t4326 (4, 4%)	0.11 (0.03-0.19)
Portugal									
ICU 1	51	5	ST22-IVh 42 (82%)	ST36-II 3 (6%)	ST105-II 3 (6%)	t747 (26, 51%)	t032 (8,16%)	t002 (5, 10%)	0.32 (0.15-0.48)
ICU 2	42	5	ST22-IVh 27 (64%)	ST36-II 10 (24%)	ST239-III 3 (7%)	t747 16 (38%)	t018 (10, 24%)	t032 (8, 19%)	0.54 (0.40-0.68)
Italy	18	6	ST228-I 8 (44%)	ST8-IVc 3 (17%)	ST36-II 3 (17%)	t041 (5, 28%)	t008 (3, 17%)	t018 (3, 17%)	0.77 (0.62-0.92)
Greece									
ICU 1	60	7	ST239-III 32 (53%)	ST225-II 14 (23%)	ST5-II 5 (8%)	t037 (32, 53%)	t003 (13, 22%)	t002 (3, 5%)	0.66 (0.55-0.76)
ICU 2	5	2	ST239-III 4 (80%)	ST30-IVc 1 (20%)	--	t037 (3, 60%)	t018 (1, 20%)	t030 (1, 20%)	0.4 (0.0-0.83)
Slovenia									
ICU 1	19	5	ST111-I 7 (37%)	ST225-II 7 (37%)	ST228-I 2 (11%)	t041 (7, 37%)	t003 (6, 32%)	t001 (2, 11%)	0.74 (0.63-0.85)
ICU 2	7	2	ST111-I 4 (57%)	ST225-II 3 (43%)	--	t003 (3, 43%)	t041 (3,43%)	t9393 (1, 14%)	0.57 (0.47-0.68)
Spain	18	8	ST8-IVc 7 (39%)	ST5-IVc 2 (11%)	ST22-IVh 2 (11%)	t008 (6, 33%)	t018 (2, 11%)	t032 (2, 11%)	0.84 (0.70-0.97)
Luxembourg	18	7	ST225-II 8 (44%)	ST710-II 4 (22%)	ST8-IVc 2 (11%)	t003 (12, 67%)	t008 (2, 11%)	t002 (1, 6%)	0.77 (0.62-0.93)
Total	500	31	ST368-III 99 (20%)	ST8-IVc 98 (20%)	ST22IVh 75 (15%)	t0425 (75, 16%)	t008 (74, 15%)	t747 (44, 9%)	0.88 (0.87-0.89)

Notes: MRSA: methicillin-resistant *Staphylococcus aureus*, ICU: intensive-care unit, pt: patient, ST: sequence type, CI: confidence interval.

five

In three ICUs from three countries (Italy, Slovenia and Luxembourg) *agr* type 2 was most prevalent. In two isolates *agr* was untypeable.

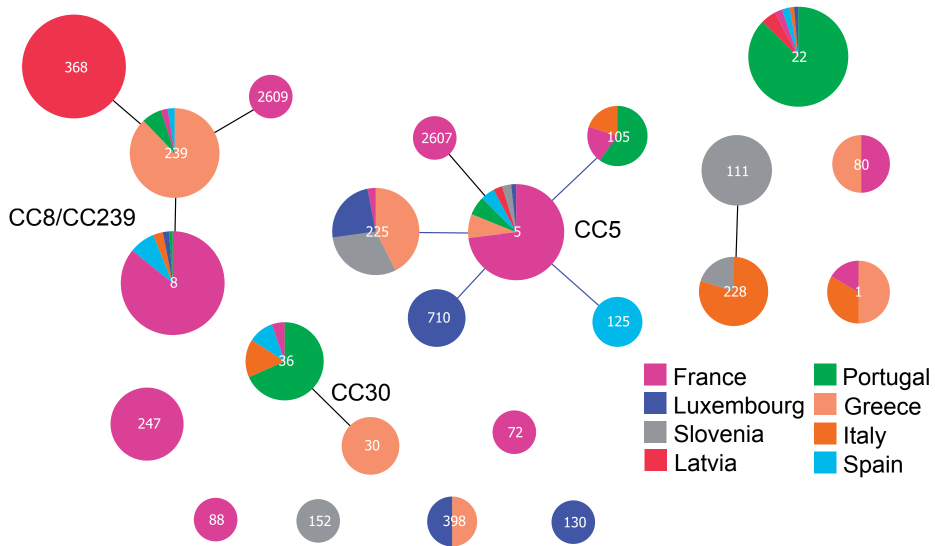


Figure 1. Clonal distribution of MRSA in European ICUs

Table 5. SCC*mec (sub)*types identified in MRSA isolates across all countries and ICUs

SCC*mec* type	n = 500 (%)
I	40 (8%)
II	69 (14%)
III	141 (28%)
IV	211 (42%)
IVa	21 (4%)
IVc	113 (23%)
IVg	1 (<1%)
IVh	75 (15%)
IV-NT	1 (<1%)
V	4 (1%)
VI	34 (7%)
XI	1 (<1%)

Note: SCC*mec:* staphylococcal cassette chromosome *mec,* NT: non-typeable.

Table 6. *Spa*-typing results of the most commonly isolated MRSA clones across all ICUs and countries and Panton Valentine leucocidin positivity in HA-MRSA, CA-MRSA and LA-MRSA clones

		***Spa* types**	***pvl***
Clone	**n=500**	**Top 3 *spa* types isolated per clone (% of MRSA clone)**	***n (%)***
HA-MRSA	480 (96%)		
ST368-III	99 (20%)	t425 (78%), t3563 (19%), t4410 (2%)	0
ST8-IVc	98 (20%)	t008 (76%), t304 (6%), t024 (4%)	0
ST22-IVh	75 (15%)	t747 (59%), t032 (25%), t020 (3%)	0
ST239-III	41 (8%)	t037 (95%), t030 (2%), t945 (2%)	0
ST5-VI	34 (7%)	t777 (97%), t179 (3%)	0
ST225-II	33 (7%)	t003 (94%), t045 (3%), t4336 (3%)	0
ST36-II	19 (4%)	t018 (95%), t012 (5%)	0
ST247-I	13 (3%)	t052 (69%), t024 (23%), t844 (8%)	0
ST111-I	11 (2%)	t041 (91%), t9393 (9%)	0
ST228-I	10 (2%)	T041 (50%), t001 (40%), t1628 (10%)	0
ST5-II	8 (2%)	t002 (63%), t895 (25%), t688 (13%)	0
Other clones	39 (8%)		1[a]
CA-MRSA	17 (3%)	t127 (35%), t044 (24%), t018 (18%)	6 (35%)[b]
LA-MRSA	3 (<1%)	t011, t899, t1736	0

Note: *pvl*: Panton-Valentine leucocidin, *agr*: accessory gene regulator, MRSA: methicillin-resistant *Staphylococcus aureus,* HA: hospital associated, CA: community-associated, LA: livestock associated. [a]*pvl* positive isolate: ST5-IVc, [b]*pvl* positive isolates: four ST80-IVc, one ST8-IVa, one ST152-V.

Community-associated MRSA and Livestock-associated MRSA

Seventeen (3%) isolates were – according to the molecular epidemiological definitions used, CA-MRSA clones, of which nine (53%) were from the Greek ICUs. Six patients carried ST1-IVa, *pvl* negative (USA400), four ST80-IVc *pvl* positive (the European clone), four ST30-IVc *pvl* negative (the South-West Pacific related clone) and three patients with either USA300 (ST8-IVa, *pvl* and ACME positive), ST 88-IVa and ST152-V, respectively. Three patients carried LA-MRSA, two from Luxembourg and one from Greece (two ST398-IVa and one ST130-XI).

Genetic diversity

The Simpson's index for genetic diversity was 0.88 (95% CI: 0.87-0.89) for all ICUs combined, without significant differences between the three study phases (pI: 0.89, 95% CI 0.87-0.91; pII: 0.90, 95% CI 0.88-0.92; pIII: 0.85, 95% CI 0.83-0.88). The index for individual ICUs ranged from 0.11 to 0.84 (Table 1) and genetic diversity was inversely correlated to acquisition rate (ß=-0.06, 95% CI: -0.10–-0.03, p=0.003) (Figure 2). Diversity was lowest in the ICU from Latvia (0.11, 95% CI: 0.03-0.19) and from Portugal (0.32, 95% CI: 0.15-0.48). No significant differences were found between ICUs within the same country.

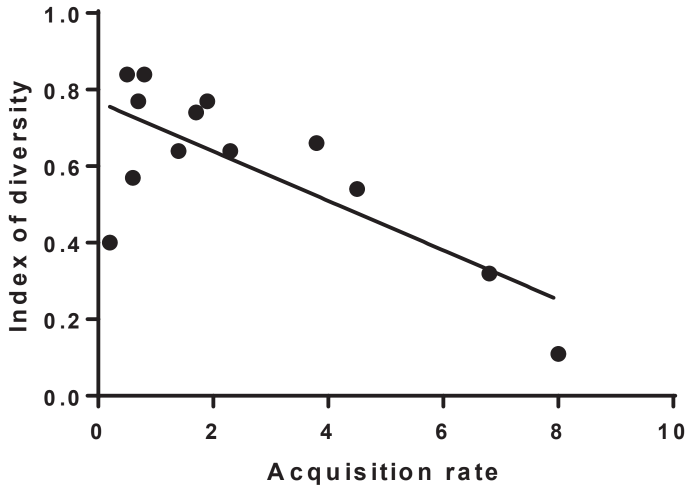


Figure 2. Relation between diversity and acquisition rate

DISCUSSION

This descriptive post-hoc analysis of a multinational prospective study in 13 European ICUs across eight countries reveals that the molecular epidemiology of MRSA was homogeneous within, but heterogeneous between countries and that CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were detected sporadically. In the MOSAR trial, the implementation of universal chlorhexidine body washing together with improving hand hygiene (phase II, six months) was associated with a statistically significant reduction of MRSA-acquisition, which persisted but did not further reduce, in phase III (12-15 months) in which admission screening and isolation of carriers was added as control measure.[8]

The homogeneity of sequence types between ICUs within the same country may result from the geospatial distribution of ICUs. Participating ICUs from France were situated in the region of Île-de-France, both hospitals from Greece were in Athens and in Slovenia both ICUs were in or close to Ljubljana. Only the two ICUs in Portugal were 100 km apart. Our findings confirm previously reported regional distribution of MRSA in Europe,[27] but extrapolation of our results of 13 individual ICUs in eight different countries is not possible.

During the two-year study period 17 patients with CA-MRSA and seven patients with *pvl* positive genotypes were identified among 8,519 patients. In 11 patients carriage with CA-MRSA or *pvl* positive genotypes was detected during the first 48 hours of ICU admission. A study evaluating the molecular epidemiology of MRSA in an Italian ICU, reported that from 62 MRSA samples typed from clinical cultures, eight (13%) were CA-MRSA. For comparison, in 18 ICUs in the United States, in which a similar surveillance

strategy as used in our study was applied in 2006, 626 of 5512 patients (11.3%) were colonized with MRSA at ICU admission, of which 30 of 210 (14%) typed isolates were considered CA-MRSA. The majority of these isolates being USA300.[28] A meta-analyses concluded that MRSA colonization at ICU admission shows global variability with 5.8%–8.3% of patients being colonized.[29] Reported colonization rates on ICU admission in Europe ranged from 4.4% (95% CI, 3.4% - 5.4%) in North and Central Europe, to 3.5% (95% CI, 1.4% – 6.7%) in Southern Europe. More recently, CA-MRSA, and particularly USA300, has become the dominant clone in some US hospitals with evidence of replacement of HA-MRSA.[4,30] The reason for the lower admission rate of CA-MRSA in these European ICUs, as compared to ICUs in the US, is not well understood, and more detailed analyses are warranted. Furthermore, little is known about the transmission capacity of different MRSA genotypes in ICUs and the hospital setting. There is evidence of lower transmissibility of CA-MRSA strains in Danish hospital settings,[31] and of LA-MRSA in Dutch hospitals.[32,33] Only three LA-MRSA were detected in the current study, which might result from the localization in urbanized areas of all participating ICUs. LA-MRSA is predominantly found in rural areas with a high density of pigs and pig farmers, mainly in subjects with professional exposure to these animals.[34]

Risk factor analysis was performed on aggregated data from all thirteen ICUs, potentially leading to a failure to detect some risk factors for individual ICUs or individual countries. Only age above 70 and hospitalization within one year prior to ICU admission were independent risk factors for MRSA colonization on admission, reflecting the dominance of HA-MRSA genotypes.

Strengths of this study include the rigorous screening using standardized methods in 13 ICUs and their local microbiology laboratories, and the centralized genotyping. The approach of surveillance of carriage, rather than investigating clinical isolates, yields a comprehensive and more complete representation of MRSA prevalence and epidemiology in the participating ICUs. We used the results of the local laboratories to calculate the MRSA prevalence on admission and MRSA acquisition rates. Eight percent of all isolates sent to the central laboratory were misclassified as MRSA by the local laboratories, of which 55% of these misclassified isolates were from swabs taken at admission and 73% came from two ICUs. Therefore, admission rates and acquisition rates as reported might be slightly overestimated. However, as only one MRSA isolates per patient was sent to the central laboratory the percentage of misclassified isolates may actually be lower. Yet, the surveillance schedule applied, including nasal and wound swabs only, may also have induced some underestimation of MRSA carriage, as screening of additional body sites, e.g. perineum and/or throat, may increase sensitivity of MRSA detection.[35]

In conclusion, the molecular epidemiology of MRSA in 13 European ICUs in eight countries appeared diverse and both CA-MRSA and LA-MRSA genotypes were rarely identified.

FUNDING

This study was supported by the European Commission under the Life Science Health Priority of the 6th Framework Program (MOSAR network contract LSHP-CT-2007-037941). The work performed at National Medicines Institute was partially supported by a grant no 934/6. PR UE/2009/7 and the Mikrobank 2 Programme from the Ministry of Science and Higher Education, Poland.

TRANSPARENCY DECLARATIONS

Nothing to declare.

REFERENCES

1. Kaye KS, Anderson DJ, Choi Y, Link K, Thacker P, Sexton DJ. The deadly toll of invasive methicillin-resistant staphylococcus aureus infection in community hospitals. *Clin Infect Dis*. 2008;46(10):1568-1577.
2. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant staphylococcus aureus and escherichia coli bacteremia: Estimating the burden of antibiotic resistance in europe. *PLoS Med*. 2011;8(10):e1001104.
3. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant staphylococcus aureus. *Lancet*. 2010;375(9725):1557-1568. doi: 10.1016/S0140-6736(09)61999-1.
4. Tenover FC, Tickler IA, Goering RV, et al. Characterization of nasal and blood culture isolates of methicillin-resistant staphylococcus aureus from patients in united states hospitals. *Antimicrob Agents Chemother*. 2012;56(3):1324-1330.
5. Johnson AP. Methicillin-resistant staphylococcus aureus: The european landscape. *J Antimicrob Chemother*. 2011;66 Suppl 4:iv43-iv48.
6. Grundmann H, Schouls LM, Aanensen DM, et al. The dynamic changes of dominant clones of staphylococcus aureus causing bloodstream infections in the european region: Results of a second structured survey. *Euro Surveill*. 2014;19(49):20987.
7. Verkade E, Kluytmans J. Livestock-associated staphylococcus aureus CC398: Animal reservoirs and human infections. *Infect Genet Evol*. 2014;21:523-530.
8. Derde LP, Cooper BS, Goossens H, et al. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: An interrupted time series study and cluster randomised trial. *Lancet Infect Dis*. 2014;14(1):31-39.
9. Gazin M, Lee A, Derde L, et al. Culture-based detection of methicillin-resistant staphylococcus aureus by a network of european laboratories: An external quality assessment study. *Eur J Clin Microbiol Infect Dis*. 2012;31(8):1765-1770.
10. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol*. 1991;29(10):2240-2244.
11. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of panton-valentine leukocidin-producing staphylococcus aureus in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29(5):1128-1132.
12. Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant staphylococcus aureus CC130 with a novel mecA homologue in humans in germany. *PLoS One*. 2011;6(9):e24360.

13. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-5448.
14. Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the genetic variability of genes encoding the RNA III-activating components agr and TRAP in a population of staphylococcus aureus strains isolated from cows with mastitis. *J Clin Microbiol*. 2002;40(11):4060-4067.
15. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant staphylococcus aureus: 'SCCmec IV multiplex'. *J Antimicrob Chemother*. 2007;60(1):42-48.
16. Milheirico C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in staphylococcus aureus. *Antimicrob Agents Chemother*. 2007;51(9):3374-3377.
17. Boye K, Bartels MD, Andersen IS, Moller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant staphylococcus aureus SCCmec types I-V. *Clin Microbiol Infect*. 2007;13(7):725-727.
18. Kondo Y, Ito T, Ma XX, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: Rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother*. 2007;51(1):264-274.
19. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother*. 2009;53(12):4961-4967.
20. Rolo J, Miragaia M, Turlej-Rogacka A, et al. High genetic diversity among community-associated staphylococcus aureus in europe: Results from a multicenter study. *PLoS One*. 2012;7(4):e34768.
21. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of staphylococcus aureus. *J Clin Microbiol*. 2000;38(3):1008-1015.
22. Diep BA, Stone GG, Basuino L, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette mec linkage: Convergence of virulence and resistance in the USA300 clone of methicillin-resistant staphylococcus aureus. *J Infect Dis*. 2008;197(11):1523-1530.
23. Fluit AC. Livestock-associated staphylococcus aureus. *Clin Microbiol Infect*. 2012;18(8):735-744.
24. David MZ, Daum RS. Community-associated methicillin-resistant staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23(3):616-687.
25. Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol*. 2001;39(11):4190-4192.
26. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: An application of simpson's index of diversity. *J Clin Microbiol*. 1988;26(11):2465-2466.
27. Grundmann H, Aanensen DM, van den Wijngaard CC, et al. Geographic distribution of staphylococcus aureus causing invasive infections in europe: A molecular-epidemiological analysis. *PLoS Med*. 2010;7(1):e1000215.
28. Nair N, Kourbatova E, Poole K, et al. Molecular epidemiology of methicillin-resistant staphylococcus aureus (MRSA) among patients admitted to adult intensive care units: The STAR*ICU trial. *Infect Control Hosp Epidemiol*. 2011;32(11):1057-1063.
29. Ziakas PD, Anagnostou T, Mylonakis E. The prevalence and significance of methicillin-resistant staphylococcus aureus colonization at admission in the general ICU setting: A meta-analysis of published studies. *Crit Care Med*. 2014;42(2):433-444.

30. Carey AJ, Della-Latta P, Huard R, et al. Changes in the molecular epidemiological characteristics of methicillin-resistant staphylococcus aureus in a neonatal intensive care unit. *Infect Control Hosp Epidemiol*. 2010;31(6):613-619.
31. Hetem DJ, Westh H, Boye K, Jarlov JO, Bonten MJ, Bootsma MC. Nosocomial transmission of community-associated methicillin-resistant staphylococcus aureus in danish hospitals. *J Antimicrob Chemother*. 2012;67(7):1775-1780.
32. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.
33. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus. *Emerg Infect Dis*. 2013;19(11):1797-1802.
34. Feingold BJ, Silbergeld EK, Curriero FC, van Cleef BA, Heck ME, Kluytmans JA. Livestock density as risk factor for livestock-associated methicillin-resistant staphylococcus aureus, the netherlands. *Emerg Infect Dis*. 2012;18(11):1841-1849.
35. Matheson A, Christie P, Stari T, et al. Nasal swab screening for methicillin-resistant staphylococcus aureus--how well does it perform? A cross-sectional study. *Infect Control Hosp Epidemiol*. 2012;33(8):803-808.

part III

DYNAMICS OF MUPIROCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* AND COAGULASE NEGATIVE STAPHYLOCOCCI

six

CLINICAL RELEVANCE OF MUPIROCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

David J. Hetem, Marc J.M. Bonten

Journal of Hospital Infection, 2013

ABSTRACT

Mupirocin is a topical antibiotic used for decolonization of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA), both in patients and in healthcare personnel, and for treatment of local skin and soft tissue infections caused by *S. aureus* and streptococcal species. Mupirocin prevents bacterial protein synthesis by inhibiting the bacterial isoleucyl-tRNA synthetase (IleRS). Low-level resistance against mupirocin, defined as minimum inhibitory concentration (MIC) of 8–256 mg/L, results from a point mutation in the native *IleRS*, and high-level resistance (MIC ≥512 mg/L) is mediated by the *mupA* (*ileS-2*) gene, located on mobile genetic elements decoding for an alternate *IleRS*. EUCAST and BSAC clinical thresholds for *S. aureus* are ≤1 mg/L for susceptible and >256 mg/L for resistant, placing the susceptible threshold at the epidemiological cut-off value (ECOFF). Isolates with MICs above the wild type (ECOFF 1 mg/L) but without a recognized resistance mechanism (MIC ≤4 mg/L) will thus be reported intermediate. Resistance to mupirocin, both high- and low-level, reduces the effectiveness of decolonizing strategies for *S. aureus* or MRSA. Low-level resistant isolates may initially be eradicated as effectively as susceptible isolates, but recolonization appears to be more usual. Increased use of mupirocin has been associated with emergence of resistance through enhanced selective pressure and cross-transmission. Unrestricted over-the-counter use and treatment of wounds and pressure sores with mupirocin are especially strongly associated with resistance. Yet emergence of mupirocin resistance following increased use has not been reported consistently, and an integrated understanding of all factors underlying the dynamics of mupirocin resistance in hospitals and communities is lacking.

INTRODUCTION

Staphylococcus aureus is an important cause of severe healthcare-associated infections worldwide, and in many parts of the world a considerable proportion of *S. aureus* isolates is resistant to many classes of antibiotics. *S. aureus* infections are associated with increased morbidity, mortality, and higher healthcare costs, especially when infections are caused by methicillin-resistant *S. aureus* (MRSA).[1,2] Moreover, increasing incidences of hospital-acquired infections caused by MRSA add to the total burden of hospital-acquired infections, rather than replacing other infections.[3] Nasal colonization with *S. aureus* is a risk factor for subsequent nosocomial *S. aureus* infection, and pre-surgical decolonization of *S. aureus* colonization has been associated with a 58% reduction in post-surgical *S. aureus* infections.[4] Topical application of mupirocin reduced rates of *S. aureus* infections by 80% and 63% for haemodialysis patients and peritoneal dialysis patients, respectively.[5] In intensive care unit (ICU) patients, with an average stay of three days, universal nasal decolonization with mupirocin and chlorhexidine body washing was associated with a 37% reduction of MRSA clinical isolates and 44% reduction of first bloodstream infection from any pathogen.[6]

Finally, mupirocin effectively reduces MRSA carriage, in patients and in colonized healthcare workers, with an estimated success rate of 90% 1 week after treatment and ~60% after a longer follow-up period.[7] This will reduce colonization pressure and cross-transmission of MRSA in healthcare settings.[8]

Mupirocin (pseudomonic acid A) is a topical antibiotic and the cornerstone of decolonization regimens for methicillin-susceptible *S. aureus* (MSSA) and MRSA, both in patients and in healthcare personnel. Furthermore, mupirocin is used for the treatment of local skin and soft tissue infections caused by *S. aureus* and streptococcal species. Resistance of *S. aureus* to mupirocin would jeopardize the efficacy of these regimens.

This review focuses on the clinical consequences of mupirocin resistance on decolonization of *S. aureus* and MRSA, and on the associations between mupirocin use and mupirocin resistance.

Mechanism of resistance

Mupirocin was first derived from *Pseudomonas fluorescens* and prevents bacterial protein synthesis by inhibiting the bacterial isoleucyl-tRNA synthetase (IleRS). It is highly active against staphylococci, streptococci and certain Gram-negative bacteria including *Haemophilus influenzae* and *Neisseria gonorrhoeae*.[9] Low-level resistance against mupirocin, defined as a minimum inhibitory concentration (MIC) of 8–256 mg/L, results from a point mutation in the native isoleucyl RNA synthetase gene, *IleRS*, and such mutations have appeared not to be associated with substantial fitness costs.[10-12] High-level resistance (MIC ≥512 mg/L) in staphylococci is mediated by the *mupA* (*ileS-2*) gene, typically located on mobile genetic elements which decode for an alternate *IleRS*.[13] High-level mupirocin

resistance in *S. aureus* is almost always mediated through acquisition of the plasmid-based *mupA*, and has been associated with multidrug resistance (resistant to ≥3 non-beta-lactam antibiotics) in MRSA.[14-17] However, the presence of *mupA* may be associated with different phenotypes. *MupA* has been detected in *S. aureus* isolates expressing low-level mupirocin resistance, possibly because of chromosomal location of *mupA*.[18,19] *S. aureus* harbouring plasmid-encoded *mupA* susceptible to mupirocin have also been reported, even without mutations in the *mupA* gene.[20,21] Plasmids carrying *mupA* have been detected in all major circulating MRSA clones, suggesting inter-clonal transfer of these plasmids.[22-24] Intra-species transfer of *mupA* between *S. epidermidis* and *S. aureus* has also been demonstrated, both *in vitro* as *in vivo* during mupirocin prophylaxis, which implies that mupirocin resistance in coagulase-negative staphylococci (CoNS) may serve as a source for resistance in *S. aureus*.[25,26] As co-resistance to gentamicin, tetracycline and macrolides may be located alongside *mupA* on the same plasmid, mupirocin treatment may offer selective pressure for antibiotics frequently used for the treatment of *S. aureus* infections.[23]

Recently, a new plasmid-mediated mechanism for high-level mupirocin resistance, *mupB*, was detected in *S. aureus*, but the prevalence of this mechanism remains to be determined.[27]

Epidemiology

Reported prevalence rates of high-level mupirocin resistance were 3% and 5% among MRSA isolates from nasal carriage and blood cultures, respectively, from 23 hospitals in the USA in 2009–2010 and 4% among 4980 MRSA isolates collected between 1995 and 2004 from 32 Canadian hospitals.[15,28] In Europe reported prevalence levels of mupirocin resistance varied widely between and within countries; from 3% for high-level resistance among MRSA isolates in Ireland in 2006–2007, 1% for high-level resistance among MRSA isolates from France in 2011–2012, to 13% (of 375 MRSA isolates) in a single hospital in Spain and 47% (of 75 *S. aureus* isolates) in Turkey.[22,29-31] In 1997 resistance rates of 2.6% were reported among MRSA isolates recovered from hospitals in 19 European countries, but more recent data are not available.[32] In nursing homes resistance rates among MRSA isolates were 5% in 2006–2009 in England and 12% in 2008–2011 in the USA.[33,34]

Reported prevalence rates of low-level resistance from single hospital studies range from zero to 80%.[35-39] Low-level resistance was rarely detected in France and Ireland, and was not detected in 319 clinical *S. aureus* isolates collected between 2000 and 2002 in Korea and in 200 clinical MRSA isolates collected between 2008 and 2009 in Pakistan.[31,38-40] In a Swiss hospital low-level resistance steadily increased from zero to 80% of clinical MRSA isolates between 1999 and 2008.[36]

Resistance in CoNS appears more widespread with 6.5% of clinical isolates being *mupA* positive in France in 2011–2012 and 22% of bloodstream CoNS isolates being *mupA* positive in a hospital from The Netherlands in 2011.[31,41]

Detection of mupirocin resistance

Multiple laboratory testing methods have been described for determining the MIC of mupirocin, including agar dilution, broth microdilution and E-test.[42-45] The Clinical and Laboratory Standards Institute (CLSI) recommends using broth microdilution or disc diffusion for screening for high-level mupirocin-resistant *S. aureus*, only differentiating between high-level resistance and the absence of high-level resistance (Table 1). CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) both recommend using 200 µg discs for detection of mupirocin resistance by disc diffusion, whereas the British Society for Antimicrobial Chemotherapy (BSAC) now recommends the use of 20 µg discs. EUCAST clinical thresholds for *S. aureus* are ≤1 mg/L for susceptible and >256 mg/L for resistant, placing the susceptible threshold at the epidemiological cut-off value (ECOFF); the Committee argues that it has not been shown that isolates with MICs of 2 mg/L do not have a significant resistance mechanism. Isolates with MICs above the wild type (ECOFF 1 mg/L) but without a recognized resistance mechanism (MIC ≤4 mg/L) will thus be reported intermediate. To date, no clinical data on the clinical relevance of *S. aureus* strains with these MIC levels (>1 and ≤4 mg/L) has been published. MIC susceptibility thresholds of BSAC coincide with EUCAST thresholds, but disc-diffusion cut-offs differ because of the 20 µg mupirocin discs used, rather than the 200 µg discs recommended by EUCAST (Table 1). Both the 20 and 200 µg discs can differentiate accurately between intermediate resistance and high-level resistance. All thresholds provided by EUCAST apply to topical nasal administration of mupirocin only.

six

Table 1. Breakpoints and interpretation of mupirocin susceptibility testing

Organisation	Method	Breakpoints and interpretation		
		Susceptible	Intermediate	Resistant
EUCAST	Disk diffusion Tablet: 200µg	≥ 30mm	18-29mm	<18mm
	MIC	≤1 mg/L	2-256 mg/L	>256 mg/L
CLSI	Disk diffusion Tablet: 200µg	Any zone = no high-level resistance		No zone = high-level resistance
	Broth microdilution Single well: 256µg	No growth = no high-level resistance		Growth = high-level resistance
BSAC	Disk diffusion Tablet: 20µg	≥ 27mm	7-26mm	<7mm
	MIC	≤1 mg/L	2-256 mg/L	>256 mg/L

Note: CLSI: Clinical and Laboratory Standards Institute, EUCAST: European committee for antimicrobial susceptibility testing, BSAC: British Society for Antimicrobial Chemotherapy, MIC: minimal inhibitory concentration.

Genotypic techniques, i.e. *mupA* PCR, for identifying high-level resistant isolates should be interpreted with care, as genotypic and phenotypic results may differ. *mupA*-positive isolates may be susceptible to mupirocin and high-level resistant may be *mupA* negative.[18]

Mupirocin usage and developing resistance

Mupirocin use can increase resistance through enhanced antibiotic pressure, more effectively selecting mupirocin-resistant strains, and through facilitating cross-transmission. Emergence of mupirocin resistance following the increased use of mupirocin has been reported repeatedly, though not consistently, and previous mupirocin exposure has been identified as a risk factor for mupirocin resistance in MRSA.[36,46-53] Moreover, reducing mupirocin use was associated with lower mupirocin resistance levels over time.[51,52] In a Brazilian hospital, mupirocin resistance levels peaked during a policy in which every patient colonized or infected with MRSA received treatment to eradicate MRSA carriage. This included the application of mupirocin to any skin wound comprising <20% of body surface. After implementing a policy to restrict mupirocin use to patients colonized but not infected with MRSA, resistance in clinical MRSA isolates fell from 65% to 15% over a period of five years.[51]

In a Swiss hospital, a hospital-wide policy to decolonize MRSA carriers with intranasal mupirocin application started in 1994, and the proportions of MRSA blood culture isolates with mupirocin resistance, mostly low-level resistance, increased from 0% in 1999 to 95% in 2005 and was 89% in 2008. The increased proportions paralleled mupirocin use in these periods.[36]

Increasing mupirocin resistance following the increased use of mupirocin has also been reported for CoNS isolated from blood in a Dutch hospital.[41] In this study all isolates were high-level resistant, *mupA* was detected in 81 of 82 (99%) isolates, and there was no evidence of clonality. High-level mupirocin resistance was associated with co-resistance to ciprofloxacin, erythromycin and clindamycin.

Emergence of mupirocin resistance in patients undergoing peritoneal dialysis and receiving topical application of mupirocin to prevent *S. aureus* infections has been reported in multiple studies, and mupirocin resistance was associated with an increased incidence of *S. aureus* exit-site infections (odds ratio: 3.16; 95% confidence interval: 1.18–8.44).[54,55] Among 32 patients on haemodialysis and receiving daily prophylactic mupirocin application at the exit-site of catheters, resistance development to mupirocin in staphylococci (*S. aureus* and CoNS) was documented in 26 (81%) during only four months of treatment.[56]

Emergence of mupirocin resistance during short-term use of mupirocin, such as during outbreak management of MRSA and MSSA, occurs infrequently.[57] In 12 studies reporting both the efficacy of mupirocin for *S. aureus* decolonization and the number of isolates acquiring mupirocin resistance, resistance was documented in six of 741 patients.[7]

Increasing resistance against mupirocin following the implementation of perioperative *S. aureus* decolonization has not yet been reported. In two large randomized controlled

trials evaluating the efficacy of perioperative *S. aureus* decolonization, resistance was not reported in one study of 917 patients, and in six of 891 patients in the other study.[4,58] However, three of these resistant *S. aureus* isolates were from patients who did not receive mupirocin during the study. Following the implementation of perioperative nasal mupirocin prophylaxis, high-level mupirocin-resistant *S. aureus* isolates were not detected and there was no trend towards increasing prevalence of low-level resistance during a period of four years in which about 7000 patients had received prophylaxis.[59]

In a Dutch hospital in which more than 20,000 patients undergoing cardiothoracic surgery were treated with mupirocin peri-operatively, mupirocin resistance was not detected – not even in clinical *S. aureus* isolates in patients developing surgical site infection.[60]

Over-the-counter availability of mupirocin in countries with high levels of community-associated MRSA has also been associated with resistance. Unrestricted, widespread use of mupirocin in the community in New Zealand was associated with an increase of mupirocin resistance among *S. aureus* isolates from almost zero in the early 1990s to 28% in 1999.[61] In Western Australia, mupirocin was frequently used empirically to treat MRSA skin and soft tissue infections in the community, and this was also associated with emergence of a mupirocin-resistant community-associated MRSA strain.[62] In response the Health Department issued guidelines recommending that mupirocin should not be used without laboratory control, that its use should not exceed 10 days, and that a patient should not have a repeat prescription within one month of completing the first course. After the implementation of these guidelines resistance dropped from 18% to 0.3% in four years.[63]

Relevance of mupirocin resistance

High-level resistance to mupirocin has been associated with failure of MRSA decolonization (Table 2).[20,64-67] When combining results from five studies, successful MRSA decolonization was achieved in 24% of 84 patients with a high-level resistant isolate, which is comparable to a decolonization rate of 29% (of 103 patients) from three studies with patients colonized with low-level resistant *S. aureus*.[18,20,64,66-69] In the five studies of patients with MRSA, decolonization was successful in 62% of 627 patients with mupirocin-susceptible *S. aureus*. Four of these studies were performed in a hospital setting and follow-up ranged from three days to one year. Naturally, differences in follow-up time between studies will influence the effectiveness of decolonization, as recolonization of *S. aureus* and MRSA tends to increase with time. Genotyping of strains may help to differentiate between recurrences and new acquisition events.[66,70]

Because of the high concentrations achieved with topical application of mupirocin, the clinical relevance of low-level resistance to mupirocin has been questioned.[68,71,72] Multiple studies evaluating the clinical relevance of low-level resistance were underpowered and low-level mupirocin was not significantly associated with eradication failure of MRSA carriage, although trends toward more failure were apparent in most studies.[66,67,69,73]

Table 2. Studies evaluating the clinical impact of mupirocin resistance

Study	Year	Study design	Patient population	MRSA / MSSA	Mupirocin S / LR / HR with follow-up available	Follow-up (days)	Eradication Mup S	Eradication Mup LR / HR	Notes
Harbarth et al.[69]	1999	DB-RCT	Hospital	MRSA	37 S, 11 LR in MUP 38 S, 12 LR in PLC	26	32% (12/37)	0% (0/11)	All patients with LR isolates in the PLC group were still colonized at follow-up
Semret et al.[68]	2001	Prospective cohort	Hospital	MRSA	16 S, 18 LR/HR	18	69% (11/16)	39% (7/18)	No differentiation was made between low-level and high-level resistance
Walker et al.[67]	2003	Prospective cohort	Hospital	MRSA	14 S, 5 LR, 18 HR	3	79% (11/14)	80% (4/5) LR 28% (5/18) HR	Eradication was achieved in only 25% LR at 14-28 days follow-up
Mody et al.[75]	2003	DB-RCT	Long term care facility	MRSA and MSSA	--	14	--	--	Four patients failed decolonization: three had resistant strains (2HR/1LR). No additional data available
Simor et al.[66]	2007	O-RCT	Hospital	MRSA	91S, 21 HR	90	74% (67/91)	24% (5/21)	No information on decolonization was provided for 5 patients with LR at baseline
Robicsek et al.[65]	2009	Retrospec-tive cohort	Hospital	MRSA	329 S, 27 HR	48 median (range 2-371)	--	--	No susceptibility tested in 51 isolates, different treatment regimes
Gilpin et al.[64]	2010	Prospective cohort	Hospital	MRSA	107 S, 23 HR	182-365	65% (69/107)	13% (3/23)	No LR identified
Lee et al.[18]	2011	Nested case control	Hospital	MRSA	75 S, 75 LR*	30-365	65% (49/75)	35% (26/75)*	68 patients with LR and 51 patients with S isolates had genotypic chlorhexidine resistance (*qacA/B* genes)
Fritz et al.[20]	2013	Prospective cohort	Community	MRSA and MSSA	324 S, 4 HR	30-365	56% (181/324)	0% (0/4)	

Note: MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-susceptible *Staphylococcus aureus*. MUP: mupirocin group, PLC: placebo group, S: susceptible, LR: low-level resistant, HR: high-level resistant, DB-RCT: double-blind randomized controlled trial, O-RCT: open randomized trial. * Save for one isolate, all isolates also harboured *qacA/B* genes encoding for chlorhexidine resistance.

In a nested case–control study of 75 patients in whom MRSA decolonization was successful and 75 patients in whom MRSA decolonization failed, the combined presence of low-level mupirocin resistance and the *qacA/B* gene associated with an elevated MIC for chlorhexidine was associated with eradication failure of MRSA carriage (odds ratio for failure: 3.4; 95% confidence interval: 1.5–5.7), as determined one to 12 months after decolonization treatment. Resistance to mupirocin and chlorhexidine was closely linked and the effects of these resistance components could not be separated. In 24% of the MRSA isolates with low-level resistance to mupirocin, *mupA* was detected alongside a point mutation, V588F, in the native *ileRS* plasmid. In univariate analysis, both *mupA* and the V588F point mutation were independently associated with decolonization failure.[18] In another study, four of five patients with low-level resistant MRSA isolates were decolonized three days after completing a five-day mupirocin eradication treatment, but recolonization occurred frequently, with only 25% of the patients remaining decolonized after 14–28 days.[67]

In conclusion, both high- and low-level resistance are associated with *S. aureus* decolonization failure. Even though sufficient data are lacking, low-level resistant isolates might initially be cleared as effectively as wild-type isolates even though recolonization is frequent. Therefore, even low-level mupirocin resistance in *S. aureus* may have implications for individual patients and hospitals, as it may lead to decolonization failure leaving the patient at increased risk for developing an endogenous *S. aureus* or MRSA infection, or allowing transmission of strains to other patients.

Future perspectives

There are three indications for mupirocin that could potentially increase its use in the near future; universal decontamination of patients admitted to ICU (as in Huang *et al.*[6]), decontamination of MRSA carriers in the community, and decolonization for perioperative prophylaxis. For the latter indication, effectiveness was demonstrated in a trial in which only *S. aureus* carriers, demonstrated with real-time polymerase chain reaction (PCR)-based testing, received mupirocin.[4] However, in the Dutch setting decolonization of all patients undergoing surgery (i.e. orthopaedic, cardiac and neurosurgery) is probably more cost-effective than screening all patients with a PCR assay prior to surgery and decolonizing only the nasal carriers of *S. aureus*.[74] The cost-effectiveness balance might be more attractive if culture-based methods were used, but these may pose a logistical challenge because of the unavoidable diagnostic delay. The downside of universal decontamination is the unnecessary use of mupirocin in 70–80% of the patients not carrying *S. aureus*, potentially enhancing resistance in CoNS and creating a reservoir of mupirocin resistance for *S. aureus*. The same holds for universal application of mupirocin in ICU patients, as might be proposed on the beneficial results of a recent multicentre study in the USA.[6] The reported co-resistance to other antibiotics in CoNS is an additional risk for patient treatment, especially in prosthetic-joint infections. Therefore, the risks of universal mupirocin prophylaxis should be carefully evaluated.

Several questions remain to be answered. First, there is a paucity of data on the prevalence of mupirocin resistance and trends in emerging resistance from European countries, and such data are especially important in countries implementing universal decolonization strategies. On a smaller scale, no data are available on the emergence of resistance against mupirocin in *S. aureus* and CoNS in individual patients undergoing decolonization treatment.

Second, the clinical relevance of the thresholds provided by EUCAST is unknown, especially of isolates with MICs just above wild-type (>1 and ≤ 4 mg/L), which are now reported as intermediate. Resistance mechanisms of these strains should be determined. For patients colonized with low-level resistant *S. aureus*, determination of the time to recolonization would provide further insights into the clinical relevance of such isolates.

CONCLUSION

Mupirocin resistance, both high and low level, reduces the effectiveness of decolonizing strategies for *S. aureus* or MRSA, and increased use of mupirocin has been associated with emerging resistance. Unrestricted use of mupirocin and treatment of wounds and pressure sores are strongly associated with increasing resistance. However, an integrated understanding of all factors underlying the dynamics in hospitals and communities of mupirocin resistance is still lacking. Therefore, monitoring for mupirocin resistance in clinical *S. aureus* and MRSA isolates is recommended in healthcare settings using universal decolonization in large patient populations. Furthermore, usage of mupirocin for wounds and pressure sores should be restricted as much as possible.

CONFLICT OF INTEREST STATEMENT

None declared.

FUNDING SOURCES

None.

APPENDIX

Data was for this review were gathered from PubMed from January 1989 to June 2013. Search terms included: Staphylococcus OR MRSA OR aureus AND Mupirocin* OR bactroban OR 'pseudomonic acid'. Only articles in English were included. Related articles were retrieved from the references.

REFERENCES

1. Kaye KS, Anderson DJ, Choi Y, Link K, Thacker P, Sexton DJ. The deadly toll of invasive methicillin-resistant staphylococcus aureus infection in community hospitals. *Clin Infect Dis*. 2008;46(10):1568-1577.
2. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant staphylococcus aureus and escherichia coli bacteremia: Estimating the burden of antibiotic resistance in europe. *PLoS Med*. 2011;8(10):e1001104.
3. Ammerlaan HS, Harbarth S, Buiting AG, et al. Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin Infect Dis*. 2013;56(6):798-805.
4. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of staphylococcus aureus. *N Engl J Med*. 2010;362(1):9-17.
5. Tacconelli E, Carmeli Y, Aizer A, Ferreira G, Foreman MG, D'Agata EM. Mupirocin prophylaxis to prevent staphylococcus aureus infection in patients undergoing dialysis: A meta-analysis. *Clin Infect Dis*. 2003;37(12):1629-1638.
6. Huang SS, Septimus E, Kleinman K, et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med*. 2013;368(24):2255-2265.
7. Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ. Eradication of methicillin-resistant staphylococcus aureus carriage: A systematic review. *Clin Infect Dis*. 2009;48(7):922-930.
8. Merrer J, Santoli F, Appere de Vecchi C, Tran B, De Jonghe B, Outin H. "Colonization pressure" and risk of acquisition of methicillin-resistant staphylococcus aureus in a medical intensive care unit. *Infect Control Hosp Epidemiol*. 2000;21(11):718-723.
9. Sutherland R, Boon RJ, Griffin KE, Masters PJ, Slocombe B, White AR. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrob Agents Chemother*. 1985;27(4):495-498.
10. Farmer TH, Gilbart J, Elson SW. Biochemical basis of mupirocin resistance in strains of staphylococcus aureus. *J Antimicrob Chemother*. 1992;30(5):587-596.
11. Antonio M, McFerran N, Pallen MJ. Mutations affecting the rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in staphylococcus aureus. *Antimicrob Agents Chemother*. 2002;46(2):438-442.
12. Hurdle JG, O'Neill AJ, Ingham E, Fishwick C, Chopra I. Analysis of mupirocin resistance and fitness in staphylococcus aureus by molecular genetic and structural modeling techniques. *Antimicrob Agents Chemother*. 2004;48(11):4366-4376.
13. Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin resistance in staphylococcus aureus J2870. *Antimicrob Agents Chemother*. 1994;38(5):1205-1208.
14. Udo EE, Jacob LE, Mathew B. Genetic analysis of methicillin-resistant staphylococcus aureus expressing high- and low-level mupirocin resistance. *J Med Microbiol*. 2001;50(10):909-915.
15. Simor AE, Stuart TL, Louie L, et al. Mupirocin-resistant, methicillin-resistant staphylococcus aureus strains in canadian hospitals. *Antimicrob Agents Chemother*. 2007;51(11):3880-3886.
16. Chaves F, Garcia-Martinez J, de Miguel S, Otero JR. Molecular characterization of resistance to mupirocin in methicillin-susceptible and -resistant isolates of staphylococcus aureus from nasal samples. *J Clin Microbiol*. 2004;42(2):822-824.
17. Cadilla A, David MZ, Daum RS, Boyle-Vavra S. Association of high-level mupirocin resistance and multidrug-resistant methicillin-resistant staphylococcus aureus at an academic center in the midwestern united states. *J Clin Microbiol*. 2011;49(1):95-100.

18. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant staphylococcus aureus carriage after decolonization therapy: A case-control study. *Clin Infect Dis*. 2011;52(12):1422-1430.
19. Ramsey MA, Bradley SF, Kauffman CA, Morton TM. Identification of chromosomal location of mupA gene, encoding low-level mupirocin resistance in staphylococcal isolates. *Antimicrob Agents Chemother*. 1996;40(12):2820-2823.
20. Fritz SA, Hogan PG, Camins BC, et al. Mupirocin and chlorhexidine resistance in staphylococcus aureus in patients with community-onset skin and soft tissue infections. *Antimicrob Agents Chemother*. 2013;57(1):559-568.
21. Driscoll DG, Young CL, Ochsner UA. Transient loss of high-level mupirocin resistance in staphylococcus aureus due to MupA polymorphism. *Antimicrob Agents Chemother*. 2007;51(6):2247-2248.
22. Perez-Roth E, Lopez-Aguilar C, Alcoba-Florez J, Mendez-Alvarez S. High-level mupirocin resistance within methicillin-resistant staphylococcus aureus pandemic lineages. *Antimicrob Agents Chemother*. 2006;50(9):3207-3211.
23. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant staphylococcus aureus. *Lancet*. 2006;367(9512):731-739.
24. Han LL, McDougal LK, Gorwitz RJ, et al. High frequencies of clindamycin and tetracycline resistance in methicillin-resistant staphylococcus aureus pulsed-field type USA300 isolates collected at a boston ambulatory health center. *J Clin Microbiol*. 2007;45(4):1350-1352.
25. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from staphylococcus epidermidis to methicillin-resistant staphylococcus aureus associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother*. 2005;56(6):1166-1168.
26. Eltringham I. Mupirocin resistance and methicillin-resistant staphylococcus aureus (MRSA). *J Hosp Infect*. 1997;35(1):1-8.
27. Seah C, Alexander DC, Louie L, et al. MupB, a new high-level mupirocin resistance mechanism in staphylococcus aureus. *Antimicrob Agents Chemother*. 2012;56(4):1916-1920.
28. Tenover FC, Tickler IA, Goering RV, et al. Characterization of nasal and blood culture isolates of methicillin-resistant staphylococcus aureus from patients in united states hospitals. *Antimicrob Agents Chemother*. 2012;56(3):1324-1330.
29. Sareyyupoglu B, Ozyurt M, Haznedaroglu T, Ardic N. Detection of methicillin and mupirocin resistance in staphylococcal hospital isolates with a touchdown multiplex polymerase chain reaction. *Folia Microbiol (Praha)*. 2008;53(4):363-367.
30. Rossney A, O'Connell S. Emerging high-level mupirocin resistance among MRSA isolates in ireland. *Euro Surveill*. 2008;13(14):8084.
31. Desroches M, Potier J, Laurent F, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant staphylococcus aureus (MRSA) in france: Emergence of a mupirocin-resistant MRSA clone harbouring mupA. *J Antimicrob Chemother*. 2013.
32. Schmitz FJ, Lindenlauf E, Hofmann B, et al. The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 european hospitals. *J Antimicrob Chemother*. 1998;42(4):489-495.
33. Horner C, Parnell P, Hall D, Kearns A, Heritage J, Wilcox M. Meticillin-resistant staphylococcus aureus in elderly residents of care homes: Colonization rates and molecular epidemiology. *J Hosp Infect*. 2013;83(3):212-218.
34. McDanel JS, Murphy CR, Diekema DJ, et al. Chlorhexidine and mupirocin susceptibilities of methicillin-resistant staphylococcus aureus from colonized nursing home residents. *Antimicrob Agents Chemother*. 2013;57(1):552-558.

35. Weir SK, Fram J, Berg G, et al. Molecular epidemiology of methicillin-resistant staphylococcus aureus isolates from patients newly identified as nasal carriers. *J Clin Microbiol*. 2012;50(10):3283-3286.
36. Lee AS, Macedo-Vinas M, Francois P, et al. Trends in mupirocin resistance in meticillin-resistant staphylococcus aureus and mupirocin consumption at a tertiary care hospital. *J Hosp Infect*. 2011;77(4):360-362.
37. Fujimura S, Watanabe A. Survey of high- and low-level mupirocin-resistant strains of methicillin-resistant staphylococcus aureus in 15 japanese hospitals. *Chemotherapy*. 2003;49(1-2):36-38.
38. Yun HJ, Lee SW, Yoon GM, et al. Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a korean hospital. *J Antimicrob Chemother*. 2003;51(3):619-623.
39. Nizamuddin S, Irfan S, Zafar A. Evaluation of prevalence of low and high level mupirocin resistance in methicillin resistant staphylococcus aureus isolates at a tertiary care hospital. *J Pak Med Assoc*. 2011;61(6):519-521.
40. O'Shea S, Cotter L, Creagh S, Lydon S, Lucey B. Mupirocin resistance among staphylococci: Trends in the southern region of ireland. *J Antimicrob Chemother*. 2009;64(3):649-650.
41. Bathoorn E, Hetem DJ, Alphenaar J, Kusters JG, Bonten MJ. Emergence of high-level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. *J Clin Microbiol*. 2012;50(9):2947-2950.
42. Swenson JM, Wong B, Simor AE, et al. Multicenter study to determine disk diffusion and broth microdilution criteria for prediction of high- and low-level mupirocin resistance in staphylococcus aureus. *J Clin Microbiol*. 2010;48(7):2469-2475.
43. de Oliveira NE, Cardozo AP, Marques Ede A, dos Santos KR, Giambiagi-deMarval M. Interpretive criteria to differentiate low- and high-level mupirocin resistance in staphylococcus aureus. *J Med Microbiol*. 2007;56(Pt 7):937-939.
44. Palepou MF, Johnson AP, Cookson BD, Beattie H, Charlett A, Woodford N. Evaluation of disc diffusion and etest for determining the susceptibility of staphylococcus aureus to mupirocin. *J Antimicrob Chemother*. 1998;42(5):577-583.
45. Finlay JE, Miller LA, Poupard JA. Interpretive criteria for testing susceptibility of staphylococci to mupirocin. *Antimicrob Agents Chemother*. 1997;41(5):1137-1139.
46. Talon D, Marion C, Thouverez M, Bertrand X. Mupirocin resistance is not an inevitable consequence of mupirocin use. *J Hosp Infect*. 2011;79(4):366-367.
47. Caierao J, Berquo L, Dias C, d'Azevedo PA. Decrease in the incidence of mupirocin resistance among methicillin-resistant staphylococcus aureus in carriers from an intensive care unit. *Am J Infect Control*. 2006;34(1):6-9.
48. Petinaki E, Spiliopoulou I, Kontos F, et al. Clonal dissemination of mupirocin-resistant staphylococci in greek hospitals. *J Antimicrob Chemother*. 2004;53(1):105-108.
49. Hogue JS, Buttke P, Braun LE, Fairchok MP. Mupirocin resistance related to increasing mupirocin use in clinical isolates of methicillin-resistant staphylococcus aureus in a pediatric population. *J Clin Microbiol*. 2010;48(7):2599-2600.
50. Miller MA, Dascal A, Portnoy J, Mendelson J. Development of mupirocin resistance among methicillin-resistant staphylococcus aureus after widespread use of nasal mupirocin ointment. *Infect Control Hosp Epidemiol*. 1996;17(12):811-813.
51. Vivoni AM, Santos KR, de-Oliveira MP, et al. Mupirocin for controlling methicillin-resistant staphylococcus aureus: Lessons from a decade of use at a university hospital. *Infect Control Hosp Epidemiol*. 2005;26(7):662-667.

52. Walker ES, Levy F, Shorman M, David G, Abdalla J, Sarubbi FA. A decline in mupirocin resistance in methicillin-resistant staphylococcus aureus accompanied administrative control of prescriptions. *J Clin Microbiol*. 2004;42(6):2792-2795.
53. Caffrey AR, Quilliam BJ, LaPlante KL. Risk factors associated with mupirocin resistance in meticillin-resistant staphylococcus aureus. *J Hosp Infect*. 2010;76(3):206-210.
54. Annigeri R, Conly J, Vas S, et al. Emergence of mupirocin-resistant staphylococcus aureus in chronic peritoneal dialysis patients using mupirocin prophylaxis to prevent exit-site infection. *Perit Dial Int*. 2001;21(6):554-559.
55. Perez-Fontan M, Rodriguez-Carmona A, Rosales M, Garcia-Falcon T, Valdes F. Incidence and clinical significance of nasal and pericatheter colonization by gram-negative bacteria among patients undergoing chronic peritoneal dialysis. *Nephrol Dial Transplant*. 2002;17(1):118-122.
56. Teo BW, Low SJ, Ding Y, Koh TH, Hsu LY. High prevalence of mupirocin-resistant staphylococci in a dialysis unit where mupirocin and chlorhexidine are routinely used for prevention of catheter-related infections. *J Med Microbiol*. 2011;60(Pt 6):865-867.
57. Ellis MW, Griffith ME, Dooley DP, et al. Targeted intranasal mupirocin to prevent colonization and infection by community-associated methicillin-resistant staphylococcus aureus strains in soldiers: A cluster randomized controlled trial. *Antimicrob Agents Chemother*. 2007;51(10):3591-3598.
58. Perl TM, Cullen JJ, Wenzel RP, et al. Intranasal mupirocin to prevent postoperative staphylococcus aureus infections. *N Engl J Med*. 2002;346(24):1871-1877.
59. Fawley WN, Parnell P, Hall J, Wilcox MH. Surveillance for mupirocin resistance following introduction of routine peri-operative prophylaxis with nasal mupirocin. *J Hosp Infect*. 2006;62(3):327-332.
60. van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of staphylococcus aureus infections in surgical patients with nasal carriage: A systematic review. *J Antimicrob Chemother*. 2008;61(2):254-261.
61. Upton A, Lang S, Heffernan H. Mupirocin and staphylococcus aureus: A recent paradigm of emerging antibiotic resistance. *J Antimicrob Chemother*. 2003;51(3):613-617.
62. Riley TV, Carson CF, Bowman RA, et al. Mupirocin-resistant methicillin-resistant staphylococcus aureus in western australia. *Med J Aust*. 1994;161(6):397-398.
63. Torvaldsen S, Roberts C, Riley TV. The continuing evolution of methicillin-resistant staphylococcus aureus in western australia. *Infect Control Hosp Epidemiol*. 1999;20(2):133-135.
64. Gilpin DF, Small S, Bakkshi S, Kearney MP, Cardwell C, Tunney MM. Efficacy of a standard methicillin-resistant staphylococcus aureus decolonisation protocol in routine clinical practice. *J Hosp Infect*. 2010;75(2):93-98.
65. Robicsek A, Beaumont JL, Thomson RB,Jr, Govindarajan G, Peterson LR. Topical therapy for methicillin-resistant staphylococcus aureus colonization: Impact on infection risk. *Infect Control Hosp Epidemiol*. 2009;30(7):623-632.
66. Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant staphylococcus aureus colonization. *Clin Infect Dis*. 2007;44(2):178-185.
67. Walker ES, Vasquez JE, Dula R, Bullock H, Sarubbi FA. Mupirocin-resistant, methicillin-resistant staphylococcus aureus: Does mupirocin remain effective? *Infect Control Hosp Epidemiol*. 2003;24(5):342-346.
68. Semret M, Miller MA. Topical mupirocin for eradication of MRSA colonization with mupirocin-resistant strains. *Infect Control Hosp Epidemiol*. 2001;22(9):578-580.
69. Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant staphylococcus aureus. *Antimicrob Agents Chemother*. 1999;43(6):1412-1416.

70. Immerman I, Ramos NL, Katz GM, Hutzler LH, Phillips MS, Bosco JA,3rd. The persistence of staphylococcus aureus decolonization after mupirocin and topical chlorhexidine: Implications for patients requiring multiple or delayed procedures. *J Arthroplasty*. 2012;27(6):870-876.
71. Watanabe H, Masaki H, Asoh N, et al. Emergence and spread of low-level mupirocin resistance in methicillin-resistant staphylococcus aureus isolated from a community hospital in japan. *J Hosp Infect*. 2001;47(4):294-300.
72. Cookson BD. The emergence of mupirocin resistance: A challenge to infection control and antibiotic prescribing practice. *J Antimicrob Chemother*. 1998;41(1):11-18.
73. Harbarth S, Liassine N, Dharan S, Herrault P, Auckenthaler R, Pittet D. Risk factors for persistent carriage of methicillin-resistant staphylococcus aureus. *Clin Infect Dis*. 2000;31(6):1380-1385.
74. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and eradication of staphylococcus aureus carriage. *PLoS One*. 2011;6(5):e14815.
75. Mody L, Kauffman CA, McNeil SA, Galecki AT, Bradley SF. Mupirocin-based decolonization of staphylococcus aureus carriers in residents of 2 long-term care facilities: A randomized, double-blind, placebo-controlled trial. *Clin Infect Dis*. 2003;37(11):1467-1474.

seven

EMERGENCE OF HIGH-LEVEL MUPIROCIN RESISTANCE IN COAGULASE-NEGATIVE STAPHYLOCOCCI ASSOCIATED WITH INCREASED SHORT-TERM MUPIROCIN USE

Erik Bathoorn, David J. Hetem, Jeriela Alphenaar, Johannes G. Kusters, Marc J. M. Bonten

Journal of Clinical Microbiology, 2012

ABSTRACT

In our hospital, mupirocin has increasingly been used for peri-operative decolonization of *Staphylococcus aureus*. The target for mupirocin is isoleucyl tRNA synthetase (*ileS*). High-level resistance to mupirocin is conferred by acquisition of plasmids expressing a distinct *ileS* gene (*ileS2*). Here we evaluated the longitudinal trends in high-level mupirocin resistance in coagulase-negative staphylococci (CoNS) and linked this to the presence of *ileS2* genes and mupirocin use. We assessed mupirocin resistance in CoNS bloodstream isolates from 2006 to 2011 tested by Phoenix automated testing (PAT). We evaluated the reliability of PAT results using Etest. PAT species determination was confirmed by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry. We investigated the presence of *ileS2* in the first 100 consecutive CoNS bloodstream isolates of each year using RT-PCR. Mupirocin use increased from 3.6 kg/year in 2006 to 13.3 kg/year in 2010 and correlated with the increase in the percentage of CoNS isolates carrying *ileS2* (8% in 2006 to 22% in 2011; Spearman's rho, 0.137; *P* = 0.01). The sensitivity and specificity of PAT for detecting high-level mupirocin resistance were 0.97 and 0.97, respectively. *ileS2* was detected in 81 of 82 phenotypically highly mupirocin-resistant strains and associated with resistance to ciprofloxacin, erythromycin, and clindamycin. In conclusion, we found a rapid increase in high-level resistance to mupirocin and resistance to other antibiotics in CoNS associated with an increase in mupirocin use. The associated resistance to other antibiotics may result in a reduction of oral antibiotic options for prolonged treatment of prosthetic infections with CoNS.

INTRODUCTION

Mupirocin is a topical antibiotic used for treatment of superficial wound infections and for eradication of nasal *Staphylococcus aureus* carriage in patients on hemodialysis or scheduled for surgery and as a measure in controlling outbreaks of methicillin-resistant *S. aureus* (MRSA). Mupirocin has bacteriostatic activity against *Staphylococcus*, *Streptococcus*, *Neisseria*, and *Haemophilus* spp.[1] through binding to isoleucyl tRNA synthetase (*ileS*), which prevents protein synthesis.[2] Most if not all wild-type staphylococcal species are susceptible to mupirocin (MICs ≤ 4 μg/ml). Mupirocin resistance occurs in two phenotypes: low-level resistance (MIC usually between 8 and 64 μg/ml) and high-level resistance (MIC ≥ 512 μg/ml).[3] Low-level resistance occurs through mutations in the native chromosomal *ileS* gene encoding isoleucyl tRNA synthetase, whereas high-level resistance is mediated by plasmids carrying the *ileS2* gene, also known as *mupA*, which encodes a novel tRNA synthetase.[4] The *ileS2* gene is detectable in nearly all highly resistant staphylococcal isolates,[5,6] and although *ileS2* does not encode resistance to other antibiotics, the presence of *ileS2*-carrying plasmids has been associated with resistance to other antibiotics, such as clindamycin, tetracycline, erythromycin, and levofloxacin.[7] Extensive and long-term use of mupirocin may facilitate the emergence of resistance to this drug,[8,9] whereas short-term intranasal use of mupirocin, as in peri-operative prophylaxis, was not associated with mupirocin resistance in clinical studies.[10] Plasmid-mediated high-level mupirocin resistance can spread clonally and horizontally, even between different staphylococcal species.[1]

Since mupirocin use has increased in our hospital, we evaluated longitudinal trends in high-level mupirocin resistance in *S. aureus* and coagulase-negative staphylococci (CoNS), the prevalence of *ileS2* genes, and mupirocin use.

MATERIALS AND METHODS

Mupirocin susceptibility was tested in all staphylococcal bloodstream isolates obtained between 2006 and 2011. Susceptibility had been tested routinely by Phoenix automated testing (PAT) (Becton, Dickinson and Company, Breda, the Netherlands). All isolates had been stored at −70°C. CoNS were distinguished from *S. aureus* by tube coagulase, DNase, and slide testing. Species determination was performed by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry (Bruker Daltonics, Bremen, Germany). Mupirocin susceptibility of the first 40 consecutive CoNS isolates of each year was also determined by Etest according to the manufacturer's guidelines on Mueller-Hinton agar (AB Biodisk, Mannheim, Germany). Susceptibility to ciprofloxacin, trimethoprim-sulfamethoxazole (TMP-SMX), erythromycin, clindamycin, tetracycline, and oxacillin was tested by PAT and interpreted according to CLSI guidelines.[11]

The presence of the *ileS2* gene was determined in the first 100 consecutive blood culture CoNS isolates of each year (from 2006 to 2011) using a Lightcycler 480 real-time

PCR system (Roche Diagnostics, Mannheim, Germany). For this, isolates were grown overnight at 37°C on sheep blood agar (Oxoid Deutschland GmbH, Wesel, Germany), and three to five colonies were suspended in 1 ml lysis buffer (Roche Diagnostics, Mannheim, Germany). The primers FW (5'-CTAAAGATTTAGGATACTGGGTTGAC) and REV (5'-GGAATGTAGATAATATATTCCATACACTTTC) (Invitrogen, Breda, the Netherlands) were designed to amplify the *ileS2* gene based on the previously described sequence.[12] Samples were heated to 95°C for 10 min. Forty-five cycles were run at 95°C for 15 s and at 60°C for 1 min. Samples were cooled to 40°C for 40 s. PCR tests were performed in duplicate. A mupirocin-susceptible *S. aureus* strain and a strain carrying the *ileS2* gene (donated by A. J. de Neeling, Laboratory for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment) were used as controls in every run; phocine herpesvirus was used as an internal control. In case of discrepancy between PCR *ileS2* gene detection and mupirocin susceptibility, PCR was repeated with susceptibility testing by Etest and species determination by MALDI-TOF mass spectrometry. We analyzed the mupirocin susceptibility of all *S. aureus* bloodstream isolates from 2006 to 2011 tested by PAT.

Mupirocin use data were provided by the Department of Clinical Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Correlations were assessed using the Spearman correlation test, and proportions were compared between groups by chi-square test. A P value of <0.05 was considered significant; a P value of <0.1 was considered to indicate a trend. Results were analyzed using SPSS 15.0.

RESULTS

The University Medical Center Utrecht (UMC Utrecht) is a 1,042-bed academic teaching hospital in the center of the Netherlands, with about 28,000 clinical and 15,000 day care hospitalizations and 334,000 outpatient visits annually. In the 5-year study period, there were 595 CoNS blood culture isolates, and the prevalence of high-level mupirocin resistance due to *ileS2* increased from 8% in 2006 to 22% in 2011 (Figure 1). The annual volume of mupirocin use increased from 3.6 kg in 2006 to 13.3 kg in 2010, which correlates with the trend in high-level resistance among CoNS (Spearman's rho, 0.137; P = 0.01). The median duration of mupirocin use per patient was 4.3 days (interquartile range, 2.5 to 5.0 days).

Only 2 of 362 *S. aureus* blood isolates collected between 2006 and 2011 were highly resistant to mupirocin.

Among 238 CoNS bloodstream isolates that were further investigated (2 isolates did not grow), *S. epidermidis* was most prevalent (n = 150, 63%), and was also the most common species with high-level resistance to mupirocin (n = 25; 78% of all isolates with high-level resistance) (Table 1). The median time from start of hospitalization to the day on which the positive blood culture was taken was 9 days.

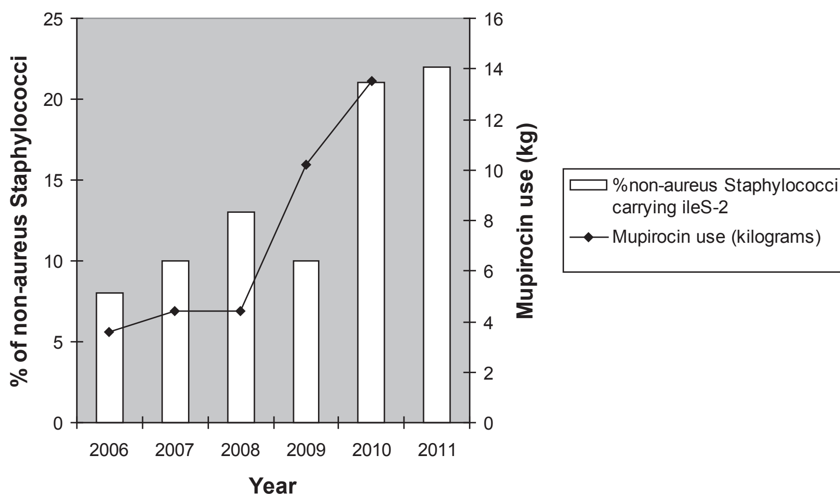


Figure 1. Correlation between emergence of high-level mupirocin resistance and increased use of mupirocin (n = 595). Histogram bars represent the percentage of CoNS bloodstream isolates carrying the *ileS2* gene and exhibiting high-level mupirocin resistance, which increased significantly from 2006 (8%) to 2011 (21%). The line shows the increase in mupirocin use from 2006 (3.6 kg) to 2010 (13.3 kg), which correlated with the increase in frequency of CoNS carrying *ileS2* (Spearman's rho, 0.137; P = 0.01).

Table 1. Distribution of CoNS species isolated from blood and their mupirocin susceptibility, as measured by Etest

Species	Total no. of isolates (n = 235)	% of isolates: Mupirocin susceptible (n = 192)	Mupirocin resistant (low) (n = 13)	Mupirocin resistant (high) (n = 30)[a]
S. capitis	32	81	16	3
S. epidermidis	150	78	5	17
S. haemolyticus	18	89	0	11
S. schleiferi	2	100	0	0
S. hominis	25	92	0	8
S. warneri	6	100	0	0
S. lugdunensis	2	100	0	0

[a]All mupirocin isolates with high-level resistance were *ileS2* positive, except for one *S. epidermidis* isolate.

Among 237 isolates tested by Etesting and PAT (two isolates did not grow, and PAT testing could not provide a result for 1 isolate), there was agreement at the level of 512 mg/liter for 230 isolates (199 with MICs of <512 mg/liter and 31 with MICs of ≥512 mg/liter). Six

isolates had MICs of <512 mg/liter by Etest but MICs of ≥512 mg/liter by PAT, and one isolate had results the other way around. When the Etest was used as a reference, both sensitivity and specificity of PAT were 0.97.

The correlation between *ileS2* gene detection with RT-PCR and MIC (cutoff, 512 mg/liter) was determined for 595 isolates (3 were reidentified as *Rothia mucilaginosa*, *Kocuria* sp., and *Micrococcus* sp., which are naturally resistant to mupirocin, and were therefore excluded from analysis; 2 isolates did not grow). In isolates with MICs of ≥512 mg/liter ($n = 85$), *ileS2* RT-PCR was negative for 1.*ileS2* PCR cycle threshold values for phenotypically highly resistant isolates ranged from 19.5 to 37.8. In 3 of 513 isolates with MICs of <512 mg/liter, *ileS2* was detected, with cycle threshold values of 29.3, 27.3, and 39.5. Isolates with high-level resistance to mupirocin were less susceptible to ciprofloxacin, clindamycin, and erythromycin (all $P < 0.05$) (Table 2).

Table 2. Susceptibility of various antibiotic classes associated with mupirocin resistance, as measured by Etest, in CoNS

	No. (%) of isolates[a]		
Susceptibility class	**Mupirocin susceptible (*n* = 192)**	**Mupirocin resistant (low) (*n* = 13)**	**Mupirocin resistant (high) (*n* = 30)**
Oxacillin susceptible	47 (24)	0 (0.0)	3 (10)
Clindamycin susceptible	108 (56)	4 (31)	8 (27)[b]
Ciprofloxacin susceptible	115 (60)	5 (38)	6 (20)[b]
Erythromycin susceptible	67 (35)	2 (15)	3 (10)[b]
TMP-SMX susceptible	115 (60)	5 (38)	13 (43)
Tetracycline susceptible	147 (77)	13 (100)	24 (80)

[a]$n = 238$. [b]$P < 0.05$, chi-square test comparing *ileS2*-positive (highly mupirocin-resistant) CoNS versus *ileS2*-negative CoNS.

DISCUSSION

We report an increase in the frequency of highly mupirocin-resistant CoNS in bloodstream isolates during the past 6 years, all linked to the presence of *ileS2*. The increase in *ileS2*-mediated high-level mupirocin resistance was weakly associated (rho = 0.14) with increased in-hospital use of mupirocin.

The occurrence of high-level mupirocin resistance in *S. aureus* has been reported from settings with over-the-counter availability of mupirocin, repeated use in peritoneal dialysis, and widespread use in the general population for nasal and skin lesions.[13] Its occurrence was rare in studies among hemodialysis patients or those receiving short-term peri-operative prophylaxis.[10] In our hospital, we found only two bloodstream *S. aureus* isolates with high-

level mupirocin resistance. However, the emergence of high-level mupirocin resistance in CoNS isolates indicates an expanding reservoir of plasmids encoding mupirocin resistance. These plasmids can, *in vitro*, be transferred from CoNS strains to other CoNS strains, to MRSA, and to restriction-deficient *S. aureus* strains.[14,15] The restriction system prevents the interchange of DNA with other bacterial species. In restriction-proficient strains, like most clinical methicillin-susceptible *S. aureus* isolates, horizontal plasmid transfer originating from CoNS strains seems less likely.[16] MRSA, and particularly the epidemic strain USA300, seems to be more susceptible to integration of plasmids carrying *ileS2*.[17] Therefore, we must be alert for the emergence of high-level mupirocin resistance in *S. aureus*.

There was 98% agreement between Phoenix and Etest susceptibility testing. The latter method has been demonstrated to be accurate and reproducible for determining high-level resistance to mupirocin.[18] Vitek-2 automated testing also had excellent agreement with PCR-based detection of the *ileS2* gene.[19] These findings demonstrate that automatic testing can be used to screen for high-level mupirocin resistance.

We used RT-PCR for detection of *ileS2*. Previous studies have evaluated conventional PCRs for detection of *ileS2* genes and found sensitivity and specificity of 100% compared to mupirocin susceptibility measurement by broth dilution.[5,6] PCR detection of *ileS2* has occurred with a few isolates which were phenotypically susceptible to mupirocin. These isolates had mutations of the *ileS2* gene and could change to highly resistant phenotypes under mupirocin pressure.[4] We found two isolates with a clearly positive PCR signal which did not display phenotypic resistance to mupirocin. A mutation in the *ileS2* gene might be an explanation for this discrepancy between the genotype and phenotype of these four isolates, but this has not been investigated.

We and others have found correlations between resistance to ciprofloxacin, TMP-SMX, doxycycline, and clindamycin and high-level mupirocin resistance.[7] This finding has important clinical consequences, as CoNS are frequent causes of prosthetic infections. Treatment of these infections consists of intravenous antibiotics, usually for 2 weeks, followed by prolonged treatment with oral antibiotics. In case of non-susceptibility to these antibiotics, long-term intravenous treatment is necessary.[20]

Several mechanisms have been described for co-resistance to mupirocin and other antibiotic classes. Plasmids carrying the *ileS2* gene are diverse in size and antibiotic resistance phenotype.[21,22] The co-occurrence of genes encoding resistance to mupirocin and other antibiotic classes on the same plasmid has been described.[23,24] Sequencing of a plasmid from the epidemic strain USA300 (pUSA03) has shown the co-occurrence of genes encoding lower susceptibility to macrolides and lincosamides.[17] Clonal spread of the strain with this plasmid could explain co-resistance.[23] The majority of high-level mupirocin-resistant strains (25 of 30) we have found were *S. epidermidis*. We typed these isolates by the method described by Johansson et al..[25] We observed some small clusters of multi-resistant isolates of the same type. However, there was no evidence for extensive clonal spread of a particular

S. epidermidis type among the resistant strains. Both the detection of the small clusters and the median time of hospitalization to the day on which the positive blood culture was taken of 9 days suggests that at least to some extent, *ileS2*-carrying isolates were acquired in the hospital. Future prospective trials may provide more conclusive answers on this. The ability of a plasmid encoding high-level mupirocin resistance to mobilize non-conjugated plasmids has also been reported.[16] Another mechanism might be selection of restriction-deficient strains. A deficiency in the restriction system results in strains being hyper susceptible to horizontal transfer of plasmids.[26] Such strains may be selected during mupirocin use and are more likely to possess DNA of plasmids encoding resistance to other antibiotic classes in addition to mupirocin resistance.

We conclude that an increase in in-hospital mupirocin use is associated with a rapid increase in high-level resistance to mupirocin and resistance to other antibiotics in CoNS. This may have direct clinical consequences in the treatment of prosthetic infections and may, in the long term, increase the risk of high-level resistance to mupirocin in *S. aureus*.

REFERENCES

1. Sutherland R, Boon RJ, Griffin KE, Masters PJ, Slocombe B, White AR. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrob Agents Chemother.* 1985;27(4):495-498.
2. Hughes J, Mellows G. Inhibition of isoleucyl-transfer ribonucleic acid synthetase in escherichia coli by pseudomonic acid. *Biochem J*. 1978;176(1):305-318.
3. Eltringham I. Mupirocin resistance and methicillin-resistant staphylococcus aureus (MRSA). *J Hosp Infect*. 1997;35(1):1-8.
4. Driscoll DG, Young CL, Ochsner UA. Transient loss of high-level mupirocin resistance in staphylococcus aureus due to MupA polymorphism. *Antimicrob Agents Chemother.* 2007;51(6):2247-2248.
5. Chaves F, Garcia-Martinez J, de Miguel S, Otero JR. Molecular characterization of resistance to mupirocin in methicillin-susceptible and -resistant isolates of staphylococcus aureus from nasal samples. *J Clin Microbiol*. 2004;42(2):822-824.
6. Simor AE, Stuart TL, Louie L, et al. Mupirocin-resistant, methicillin-resistant staphylococcus aureus strains in canadian hospitals. *Antimicrob Agents Chemother*. 2007;51(11):3880-3886.
7. Han LL, McDougal LK, Gorwitz RJ, et al. High frequencies of clindamycin and tetracycline resistance in methicillin-resistant staphylococcus aureus pulsed-field type USA300 isolates collected at a boston ambulatory health center. *J Clin Microbiol*. 2007;45(4):1350-1352.
8. Boyce JM. Preventing staphylococcal infections by eradicating nasal carriage of staphylococcus aureus: Proceeding with caution. *Infect Control Hosp Epidemiol*. 1996;17(12):775-779.
9. Miller MA, Dascal A, Portnoy J, Mendelson J. Development of mupirocin resistance among methicillin-resistant staphylococcus aureus after widespread use of nasal mupirocin ointment. *Infect Control Hosp Epidemiol*. 1996;17(12):811-813.
10. van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing staphylococcus aureus infections in nasal carriers. *Cochrane Database Syst Rev*. 2008;(4)(4):CD006216.
11. CLSI 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement; M100-S19, vol. 29, no. 3.

12. Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin resistance in staphylococcus aureus J2870. *Antimicrob Agents Chemother*. 1994;38(5):1205-1208.
13. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. *Clin Infect Dis*. 2009;49(6):935-941.
14. Bastos MC, Mondino PJ, Azevedo ML, Santos KR, Giambiagi-deMarval M. Molecular characterization and transfer among staphylococcus strains of a plasmid conferring high-level resistance to mupirocin. *Eur J Clin Microbiol Infect Dis*. 1999;18(6):393-398.
15. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from staphylococcus epidermidis to methicillin-resistant staphylococcus aureus associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother*. 2005;56(6):1166-1168.
16. Udo EE, Jacob LE. Conjugative transfer of high-level mupirocin resistance and the mobilization of non-conjugative plasmids in staphylococcus aureus. *Microb Drug Resist*. 1998;4(3):185-193.
17. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant staphylococcus aureus. *Lancet*. 2006;367(9512):731-739.
18. Palepou MF, Johnson AP, Cookson BD, Beattie H, Charlett A, Woodford N. Evaluation of disc diffusion and etest for determining the susceptibility of staphylococcus aureus to mupirocin. *J Antimicrob Chemother*. 1998;42(5):577-583.
19. Malaviolle X, Nonhoff C, Denis O, Rottiers S, Struelens MJ. Evaluation of disc diffusion methods and vitek 2 automated system for testing susceptibility to mupirocin in staphylococcus aureus. *J Antimicrob Chemother*. 2008;62(5):1018-1023.
20. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med*. 2004;351(16):1645-1654.
21. Janssen DA, Zarins LT, Schaberg DR, Bradley SF, Terpenning MS, Kauffman CA. Detection and characterization of mupirocin resistance in staphylococcus aureus. *Antimicrob Agents Chemother*. 1993;37(9):2003-2006.
22. Rahman M, Connolly S, Noble WC, Cookson B, Phillips I. Diversity of staphylococci exhibiting high-level resistance to mupirocin. *J Med Microbiol*. 1990;33(2):97-100.
23. Diep BA, Chambers HF, Graber CJ, et al. Emergence of multidrug-resistant, community-associated, methicillin-resistant staphylococcus aureus clone USA300 in men who have sex with men. *Ann Intern Med*. 2008;148(4):249-257.
24. Needham C, Rahman M, Dyke KG, Noble WC. An investigation of plasmids from staphylococcus aureus that mediate resistance to mupirocin and tetracycline. *Microbiology*. 1994;140 (Pt 10)(Pt 10):2577-2583.
25. Johansson A, Koskiniemi S, Gottfridsson P, Wistrom J, Monsen T. Multiple-locus variable-number tandem repeat analysis for typing of staphylococcus epidermidis. *J Clin Microbiol*. 2006;44(1):260-265.
26. Corvaglia AR, Francois P, Hernandez D, Perron K, Linder P, Schrenzel J. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical staphylococcus aureus strains. *Proc Natl Acad Sci U S A*. 2010;107(26):11954-11958.

eight

ACQUISITION OF HIGH-LEVEL MUPIROCIN RESISTANCE IN CONS FOLLOWING NASAL DECOLONIZATION WITH MUPIROCIN

David J. Hetem, H. Charles Vogely, Tim T. Severs, Annet Troelstra,
Johannes G. Kusters, Marc J. M. Bonten

Journal of Antimicrobial Chemotherapy, 2015

ABSTRACT

Objectives: The association between mupirocin use and plasmid-based high-level resistance development mediated through *mupA* in CoNS has not been quantified. We determined acquisition of mupirocin resistance in *Staphylococcus aureus* and CoNS in surgery patients treated peri-operatively with mupirocin.

Patients and methods: Patients admitted for surgery were treated with nasal mupirocin ointment and chlorhexidine soap for 5 days, irrespective of *S. aureus* carrier status. Nasal swabs were obtained before decolonization (T1) and 4 days after surgery (T2) and were inoculated onto agars containing 8 mg/L mupirocin. Staphylococci were identified by MALDI-TOF MS and mupirocin resistance was confirmed by Etest.

Results: Among 1578 surgical patients, 936 (59%) had nasal swabs obtained at T1 and T2; 192 (21%) patients carried mupirocin-resistant CoNS at T1 and 406 (43%) at T2 (P<0.001). Of 744 patients not colonized at T1, 277 acquired resistance (37%), corresponding to an acquisition rate of 7.4/100 patient days at risk. In all, 588 (97%) of 607 mupirocin-resistant CoNS had an MIC >256 mg/L (high level) and 381 of 383 (99.5%) were *mupA* positive. No acquisition of mupirocin resistance was observed in *S. aureus*.

Conclusions: Acquisition of mupirocin resistance following decolonization was widespread in CoNS and absent in *S. aureus*. As almost all isolates harboured the *mupA* gene, monitoring resistance development in *S. aureus* when decolonization strategies containing mupirocin are used is recommended.

INTRODUCTION

Mupirocin is a topical antibiotic and the cornerstone of decolonization regimens for MSSA and MRSA in patients and healthcare personnel. Peri-operative eradication of *Staphylococcus aureus* nasal carriage, with mupirocin and chlorhexidine body washings, reduces the incidence of post-operative *S. aureus* infections by 58%.[1] However, the rapid identification of *S. aureus* carriers and immediate application of treatment is logistically challenging and costly. Therefore, universal peri-operative decolonization, irrespective of *S. aureus* carrier status, would be more cost-effective.[2]

Yet, extensive use of mupirocin, as in universal decolonization, may facilitate emergence of mupirocin resistance in *S. aureus* and CoNS, but this risk has not been quantified. We therefore investigated the effects of universal decolonization with topical mupirocin and chlorhexidine body washings on resistance in CoNS and *S. aureus*.

PATIENTS AND METHODS

Setting and patient population

This study was performed at a tertiary teaching hospital in Utrecht, The Netherlands. A universal decolonization strategy was implemented on three surgical wards: a cardiothoracic surgery ward, an orthopaedic surgery ward and a neurosurgical ward for all patients undergoing surgery with an expected stay of ≥4 days, using mupirocin nasal ointment three times daily for 5 days and chlorhexidine body washings once daily for 5 days, starting decolonization on the day of admission. On the three surgical wards, nasal swabs for detection of mupirocin-resistant staphylococci were taken from all consecutive patients treated with mupirocin, between June 2012 and June 2013, before the start of decolonization treatment (T1) and 4 days after surgery (1 day after completing decolonization treatment) (T2). A nasal swab was also obtained if patients were discharged or transferred to another hospital before completing the 5 days of decolonization therapy. The monitoring of resistance on the surgical wards was approved by the infection prevention committee of the University Medical Center Utrecht and regarded as quality control (monitoring resistance) of an existing policy.

Patients able to give informed consent in the geriatric ward were approached for study participation between May 2013 and December 2013. From these patients (who did not receive decolonization), swabs were also obtained on admission (T1) and on the fifth day after admission (T2). The inclusion of the control group on the geriatric ward was approved by the medical research ethics committee of the University Medical Center Utrecht (protocol number 13-036/C).

Microbiology

Nasal swabs were collected and inoculated onto selective agars, both with and without 8 mg/L mupirocin. Species determination was performed by MALDI-TOF MS (Bruker

Daltonics, Bremen, Germany) on all suspect growing colonies. Resistance to mupirocin in growing staphylococcal isolates was confirmed by Etest (bioMérieux). The *mupA* gene was detected in staphylococcal isolates growing on the selective agars using a Lightcycler 480 II real-time PCR system (Roche Diagnostics, Mannheim, Germany). Multilocus variable tandem repeat analysis (MLVA) was used for the genotyping of *Staphylococcus epidermidis* as described in more detail elsewhere.[3] From each of the three wards, 25 consecutively isolated *S. epidermidis* strains from patients were typed. Further details on the microbiological testing, including the breakpoints used, can be found in the Supplementary data.

RESULTS

During the study period, 1578 surgical and 22 geriatric patients were screened on admittance, from whom in 936 (59%) surgical and 17 (77%) geriatric patients nasal swabs were obtained both at T1 and T2. Subsequent analysis was performed on the 936 surgical patients (930 unique patients and 6 readmissions) and 17 control patients with swabs taken at both T1 and T2. In surgical patients, mupirocin-resistant CoNS were detected at T1 and T2 in 192 (21%) and 406 (43%) patients, respectively ($P<0.001$) (Table 1). Of the 744 patients not colonized at T1 and thus at risk of acquisition of mupirocin-resistant staphylococci, 277 (37%) acquired colonization at T2. This corresponds to an acquisition rate of 7.4/100 patient days at risk. Of the 192 patients colonized with mupirocin-resistant CoNS at T1, 129 (67%) were still colonized at T2 and 63 (33%) no longer had detectable colonization at T2. All 13 patients colonized at admittance with an intermediate resistant CoNS (MIC range 8–256 mg/L) lost colonization by intermediate isolates at T2. Six of these 13 patients acquired a high-level resistant strain. None of the 17 geriatric patients was colonized with mupirocin-resistant CoNS at T1 or T2.

Overall, 607 mupirocin-resistant CoNS were identified in 469 patients, of which 588 (97%) had high-level resistance (MIC ≥512 mg/L). Almost all were *S. epidermidis* (568/607, 94%). Mupirocin-resistant *S. aureus* were not detected in 939 patients with swabs taken at T1 and T2. One patient carried a mupirocin-resistant *S. aureus* (MIC 512 mg/L) at admission, with no swab taken at T2. Antibiotic susceptibility patterns besides mupirocin were determined in 100 randomly selected CoNS, revealing co-resistance to oxacillin in 69%, aminoglycosides (either tobramycin or gentamicin resistance) in 61%, clindamycin in 61%, ciprofloxacin in 62%, trimethoprim/sulfamethoxazole in 51% and rifampicin in 8%.

A PCR for the detection of *mupA* was performed on 383/607 (63%) strains. *mupA* was detected in 381 of 383 strains (99.5%) high-level mupirocin-resistant CoNS and in four of 14 (29%) CoNS with intermediate resistance, with MICs ranging from 8 to 128 mg/L in *mupA*-positive strains. MLVA typing of 75 isolated high-level resistant *S. epidermidis* strains from the three surgery wards yielded 15 different genotypes, without a major dominant clone or evidence of clonal spread among those who acquired mupirocin-resistant *S. epidermidis* (Table S1).

Table 1. Main results: detection of mupirocin-resistant CoNS in 936 surgical patients with nasal samples taken at T1 and T2

	Mupirocin-resistant CoNS	
	T1	T2
All patients (*n*=936)	192	406
high-level resistance	179	400
intermediate resistance	13	6
Patients without colonization at T1 (*n*=744)	0	277
high-level resistance at T2	0	273
intermediate resistance at T2	0	4
Patients colonized with mupirocin-resistant CoNS at T1	192	129

DISCUSSION

In this prospective cohort study, the prevalence of high-level resistance against mupirocin among CoNS was 21% before surgery and this increased to 43% after topical treatment with mupirocin and chlorhexidine body washing. Mupirocin-resistant *S. aureus* was only detected in one of 1578 patients at admission and no acquisition of mupirocin resistance was observed.

In this study, we screened for mupirocin-resistant staphylococci with an MIC ≥16 mg/L, thereby ignoring intermediate or low-level resistant isolates with MICs <16 mg/L. Low-level mupirocin resistance in *S. aureus* is mediated through mutations in the native *ileRS*, whereas the plasmid-based *mupA* gene encodes high-level resistance. We focused on *mupA*-positive staphylococci because of the potential of this gene to spread between species *in vitro* and *in vivo*.[4,5] Because 99.5% of the high-level resistant isolates harboured *mupA*, we did not look for the presence of *mupB*, an alternative gene encoding mupirocin resistance.[6] In addition to *mupA*, the *qacA/B* genes encoding resistance to antiseptics could potentially provide an additional source for cross-transmission between staphylococcal species and are commonly found in conjunction with low-level mupirocin resistance.[7]

Clonal dissemination of mupirocin-resistant *S. aureus* and CoNS in hospital settings has been described.[8] Based on our MLVA typing, we were able to exclude the possibility that cross-transmission fuelled the acquisition during treatment. *mupA* has been detected in all major dominant MRSA clones, suggesting that interclonal transfer of *mupA* could contribute to the spread of mupirocin resistance.[9]

This study has several limitations. First, no broth enrichment was used, potentially underestimating the prevalence of *S. aureus*. Second, only a short period of follow-up was available for all patients, potentially underestimating the transfer rate of mupirocin resistance from CoNS to *S. aureus*. However, the universal decolonization strategy had

already been implemented for almost a year on the cardiothoracic ward and only one mupirocin-resistant *S. aureus* was found. Third, no typing was performed on strains from patients colonized both at T1 and T2. Finally, as mupirocin-susceptible *S. aureus* and CoNS were not collected, we could not differentiate whether acquisition of mupirocin-resistant CoNS during decolonization resulted from resistance development of the colonizing strain, from selection of a pre-existent mupirocin-resistant strain under antibiotic pressure or from exogenous acquisition. We could also not assess the percentage of patients with nasal co-carriage of mupirocin-resistant CoNS and mupirocin-susceptible *S. aureus* at admission.

Transfer of *mupA* between *S. epidermidis* and *S. aureus* has been described during mupirocin prophylaxis *in vitro* and *in vivo*.[4] However, no mupirocin-resistant *S. aureus* were found at T2 during the study period, suggesting that horizontal transmission of the *mupA* gene from CoNS to *S. aureus* did not occur in ≥38 patients (assuming 20% of the patients are *S. aureus* carriers) carrying resistant CoNS at the start of treatment and with follow-up cultures obtained. Furthermore, no acquisition of mupirocin resistance in *S. aureus* was observed, which was in stark contrast to 37% of the patients acquiring mupirocin resistance in CoNS. The differences in acquisition rates between *S. aureus* and *S. epidermidis* are poorly understood and should be studied in future work.

Based on its beneficial cost-effectiveness profile, we recommend universal decolonization with mupirocin nasal ointment and chlorhexidine body washing. However, the pre-existing high prevalence and rapid increase of plasmid-based high-level mupirocin resistance in CoNS during treatment warrant careful monitoring of mupirocin resistance development. In our study, horizontal gene transfer of *mupA* to *S. aureus* was not demonstrated in 936 patients during the 5 days of follow-up. Future studies determining the persistence of carriage with high-level mupirocin-resistant CoNS and quantifying the horizontal transfer rate in patients with longer follow-up are needed for more accurate assessment of the ecological safety of universal decolonization with mupirocin in surgical and critically ill patients.

FUNDING

This study was carried out as part our routine work and was performed using departmental funds. We received no grant from any funding agency in the public, commercial or not-for-profit sectors.

TRANSPARENCY DECLARATIONS

None to declare.

REFERENCES

1. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of staphylococcus aureus. *N Engl J Med*. 2010;362(1):9-17.
2. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and eradication of staphylococcus aureus carriage. *PLoS One*. 2011;6(5):e14815.
3. Johansson A, Koskiniemi S, Gottfridsson P, Wistrom J, Monsen T. Multiple-locus variable-number tandem repeat analysis for typing of staphylococcus epidermidis. *J Clin Microbiol*. 2006;44(1):260-265.
4. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from staphylococcus epidermidis to methicillin-resistant staphylococcus aureus associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother*. 2005;56(6):1166-1168.
5. Eltringham I. Mupirocin resistance and methicillin-resistant staphylococcus aureus (MRSA). *J Hosp Infect*. 1997;35(1):1-8.
6. Seah C, Alexander DC, Louie L, et al. MupB, a new high-level mupirocin resistance mechanism in staphylococcus aureus. *Antimicrob Agents Chemother*. 2012;56(4):1916-1920.
7. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant staphylococcus aureus carriage after decolonization therapy: A case-control study. *Clin Infect Dis*. 2011;52(12):1422-1430.
8. Petinaki E, Spiliopoulou I, Kontos F, et al. Clonal dissemination of mupirocin-resistant staphylococci in greek hospitals. *J Antimicrob Chemother*. 2004;53(1):105-108.
9. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in staphylococcus aureus. *J Hosp Infect*. 2013;85(4):249-256.

SUPPLEMENTARY DATA

Microbiology

Nasal swabs were collected using Copan Liquid Amies Elution Swab (ESwab) collection and transport system (Copan, Brescia Italy) and inoculated on selective colistin nalidixic acid (CNA) agars, both with and without 8mg/L mupirocin. Agars were inoculated for 48 hours at 37°C. Species determination was performed by MALDI-TOF (Bruker Daltonics, Bremen, Germany) on all suspect growing colonies. Resistance for mupirocin in growing staphylococcal isolates was confirmed by Etest (Biomerieux) according to the manufacturer's guidelines on Mueller-Hinton agar (AB Biodisk, Mannheim, Germany). Currently, no breakpoints exist for mupirocin resistance in CoNS, and therefore the MIC breakpoints for CoNS were based on EUCAST breakpoints for *S. aureus*. according to the manufacturer's guidelines on Mueller-Hinton agar (AB Biodisk, Mannheim, Germany). Susceptibility testing to antibiotics other than mupirocin was performed by Phoenix automated testing (Becton, Dickinson and Company, Breda, the Netherlands) using EUCAST breakpoints.

The presence of the *mupA* gene, encoding for plasmid based high-level mupirocin resistance, was determined in staphylococcal isolates by a *mupA* specific real-time PCR. For this the isolates were grown overnight at 37°C on sheep blood agar (Oxoid Deutschland GmbH, Wesel, Germany), and a single colony was suspended in 1 ml of lysis buffer (Cobas® PCR Female Swab Sample Kit, Roche Molecular Systems, Inc. Branchburg, NJ, USA), followed by freezing at -80°C for 18 h and immediate heating at 95°C for 15 min in order to liberate the DNA and to inactivate DNAses. DNA was then automatically extracted from this 100 ul sample on MagNA Pure96 (Roche), using the Roche MagNA

Table S1. MLVA typing of 75 subsequently isolated *S. epidermidis* strains from the cardio-thoracic ward

Type	N	SE1	SE2	SE3	SE4	SE5
a	31	900	450	450	400	1500
b	9	400	450	450	350	
c	8	500	450	350	400	1500
d	7	400	450	400	450	1500
e	4	900	400	400	250	500
f	3	800	400	500	400	
g	2	500	450	400	300	1200
h	2	700	400	400	400	1500
i	2	900	500	400	400	1200
j	2	400	450	400	400	250
Singletons	5					

Note: SE1-5: *Staphylococcus epidermidis* primer 1-5.

Pure96 and Viral NA Small Volume Kit with the Viral NA Universal SV extraction protocol according to the manufacturer's instructions). Phocine herpes virus was added to each sample as an internal/processing control in a concentration that was experimentally determined to result in an Cq value of ~35.[1] Final elution was in 100 µl and 2 µl of these eluates was used as input for a 15 ul PCR reaction. For this PCR the primers forward (GAATGGCGGGATTTTTCTAAAG), reverse (GGAATGTAGATAATATATTCCATACACTTTC), and probe (FAM-ATACTGGGTTGACATGGACTCCC-BBQ) (TIB Molbiol, Berlin, Germany) were used. Samples were heated to 95°C for 10 min. for Taq activation and subsequently Forty-five cycles were run (95°C for 15 s and at 60°C for 1 min.) on a Roche Lightcycler 480 II (Roche, Almere the Netherlands).

REFERENCES

1. HGM Niesters Clinical virology in real time. *J Clin Virol* 2002; 25, Suppl 3: S3-12

nine

PREVENTION OF SURGICAL SITE INFECTIONS: DECONTAMINATION BASED ON PRE-OPERATIVE SCREENING FOR *STAPHYLOCOCCUS AUREUS* CARRIERS OR UNIVERSAL DECONTAMINATION?

David J. Hetem, Martin C.J. Bootsma, Marc J.M. Bonten

Submitted

ABSTRACT

Peri-operative decolonization of *Staphylococcus aureus* nasal carriers with mupirocin together with chlorhexidine body washing reduces the incidence of *S. aureus* surgical site infection (SSI). A targeted strategy, applied in *S. aureus* carriers only, is costly and implementation may reduce effectiveness. Universal decolonization is more cost-effective, but increases exposure of non-carriers to mupirocin, and the risk of resistance to mupirocin in staphylococci. High-level mupirocin resistance in *S. aureus* can emerge through horizontal gene transfer originating from coagulase-negative staphylococci (CoNS) and through clonal transmission. The current evidence on the occurrence of high-level mupirocin resistance in *S. aureus* and CoNS, in combination with the results of mathematical modelling, strongly suggest that the increased selection of high-level mupirocin resistance in CoNS does not constitute an important risk for high-level mupirocin resistance in *S. aureus*.

As compared to a targeted strategy, universal decolonization seems associated with an equally low risk of mupirocin resistance in *S. aureus*.

BACKGROUND

Nasal carriage with *Staphylococcus aureus* occurs persistently in 20% and intermittently in 30% of human subjects.[1] Nasal *S. aureus* carriage is associated with an estimated 5-10 fold risk of developing *S. aureus* surgical site infection (SSI),[2,3] and more than 80% of all *S. aureus* SSI are thought to be from endogenous origin; i.e., caused by the same strain that previously colonized the nares.[1] Eradication of *S. aureus* carriage peri-operatively reduces the incidence of *S. aureus* SSI. Although several studies failed to demonstrate significant reductions in *S. aureus* SSI when using universal peri-operative intranasal application of mupirocin,[4] the intervention appeared beneficial in those patients that were carrying *S. aureus* pre-operatively, as determined in a meta-analysis.[5] This preventive effect was subsequently confirmed in a randomized placebo-controlled multi-center trial of targeted decolonization of *S. aureus* carriers in the Netherlands.[6] In this study application of mupirocin nasal ointment twice daily and daily chlorhexidine body washing for a total of 5 days reduced the incidence of *S. aureus* SSI by 58% and even by 79% for deep SSI, in nasal *S. aureus* carriers identified pre-operatively using PCR-based testing.[6] In a pragmatic multicenter study in the United States implementation of a bundle consisting of *S. aureus* screening and decolonization, and targeted prophylaxis reduced the number of post-operative complex *S. aureus* SSI with 42% with 39% full and 44% partial bundle adherence.[7] Incidences of complex *S. aureus* SSI rates did not decrease significantly in patients undergoing urgent or emergency surgery, or in patient groups with only partial or non-adherence to the bundle.

Implementing a screening and targeted decolonization strategy in daily practice, either with rapid molecular techniques or conventional cultures, is complicated. Only patients undergoing elective surgery can be screened in outpatient settings, and screening results need to be communicated in time, followed by allocating and administering the appropriate therapy. This may be difficult, especially when the window of opportunity before surgery is small. A too long period between screening and surgery increases the risk of misclassification. Although successful screening and initializing treatment of all eligible patients has been reported to be as high as 85%,[8,9] others have reported logistical challenges and concerns about associated costs of rapid screening.[8,10] Failure to obtain nasal samples, or to report screening results in time, or to apply medication in time will all reduce the effectiveness of this intervention, as *S. aureus* carriers may not receive treatment. Moreover, reported sensitivities of PCR-based screening have ranged from 65% to 97%, which also may lead to missing *S. aureus* carriers.[6,11,12] Yet, negative screening results have been associated with lower colonization density,[12] possibly reflecting lower infection risks.[13,14] Finally, pre-operative screening for *S. aureus* is usually performed based on nasal swabs only, which may also lead to misclassification. Indeed, screening for nasal carriage has been consistently reported to detect only 65% to 75% of methicillin-resistant *S. aureus* (MRSA) carriers,[15] but whether this also applies to methicillin-sensitive *S. aureus* (MSSA) remains unknown.

The logistical challenges and costs of pre-operative screening and the risk of not allocating a beneficial preventive measure to *S. aureus* carriers can be minimized by treating all patients, irrespective of *S. aureus* carrier status, with mupirocin and chlorhexidine body washing (Table 1). With this approach all patients, also those that would have had false negative screening results, will be treated. This also implies that all patients receive some form of protection against acquiring *S. aureus* after screening, for instance through cross-transmission during the first five days after surgery. Yet, decolonization of non-carriers will not contribute to the beneficial effects of decolonization, and although cost savings can be realized by avoiding screening, more costs will be made for treatment. Nevertheless, in cost-effectiveness modeling studies universal decolonization strategies had the highest cost savings when compared to targeted decolonization strategies, mainly because of absence of screenings costs and the higher efficacy of the intervention.[10] Importantly, though, this cost-effectiveness analysis assumed a persistent efficacy over time neglecting the potential development of resistance against any of the components of the strategy and subsequent reduction of efficacy.

Concerns exist that widespread use of mupirocin, as used in universal decolonization, will increase the risk for the development of resistance. Emergence of resistance against mupirocin has been associated, though not consistently, with an increased use of mupirocin.[16-18] Especially, unrestricted, widespread use of mupirocin in the community and the use in wounds and pressure sores have been associated with the emergence of resistance.[16,19,20] Here, we review the current evidence on the risks of developing mupirocin

Table 1. Characteristics of targeted and universal peri-operative decolonization strategies

	Universal decolonization	Targeted screening and decolonization
Implementation of strategy	Easy Prescription of medication	Logistics can/will be challenging Screening Reporting of results Prescription of medication
Sensitivity of strategy	100% (*S. aureus* carriers will not be missed)	Sub-optimal (Not all patients may be screened, test procedure may not have 100% sensitivity and non-nasal *S. aureus* carriers may be missed)
Volume of mupirocin use	Approximately five times more than in targeted strategy	Detected *S. aureus* carriers only
Volume of screening	Absent	All subjects
Cost components	 Allocation of medication Mupirocin	Screening Reporting Allocation of medication Mupirocin

resistance for two different peri-operative decolonization strategies: targeted screening and decolonization of identified *S. aureus* carriers and universal decolonization irrespective of carrier status. We use a mathematical model and available epidemiological data to explore the dynamics of mupirocin resistance within a hospital setting and to identify the most important determinants for emergence of mupirocin resistance in *S. aureus*.

Resistance to mupirocin

Mupirocin is a topical antibiotic that prevents bacterial protein synthesis by inhibiting the bacterial isoleucyl-tRNA synthetase (IleRS). It is the cornerstone for the decolonization of *S. aureus* including MRSA in both patients and health-care workers. High-level mupirocin resistance is mediated through the plasmid based *mupA* gene encoding for an alternate *ileRS*, whereas low-level resistance results from point mutation in the native *ileRS* gene. Increasing resistance against mupirocin in *S. aureus* would greatly threaten the effectiveness of these decolonization strategies, as mupirocin resistance is associated with high failure rates. Successful decolonization of subjects carrying high-level mupirocin resistant MRSA has been reported to be as low as 24%.[21] Isolates with low-level resistance appear to be initially cleared as effectively as susceptible strains, but recolonization appears to occur more frequently.[22] Several studies have quantified the development of mupirocin resistance in *S. aureus* following the implementation of decolonization strategies. In a Dutch multicenter trial resistance against mupirocin was not detected in 917 patients carrying *S. aureus* before receiving mupirocin treatment, neither was mupirocin resistance detected in any of the *S. aureus* isolates causing hospital-acquired infections.[6] Moreover, no infections (or carriage) caused by mupirocin resistant *S. aureus* were detected among more than 20,000 patients that were treated peri-operatively with mupirocin and chlorhexidine in a single Dutch hospital, not even in those patients developing post-operative *S. aureus* SSI.[5] In the United States only one of 36 (2.8%) isolates causing complex *S. aureus* SSI in *S. aureus* carriers receiving peri-operative decolonization with mupirocin and chlorhexidine body washings was high-level resistant to mupirocin.[7] In another randomized study comparing mupirocin to placebo in 871 patients in the united States, six of 1,021 *S. aureus* isolates (0.6 percent), obtained from six patients, were resistant to mupirocin during the four-year study period. It remained unknown whether resistance occurred after mupirocin exposure, but three of the six patients had not received mupirocin during the study period.[4] In a study of more than 7,000 patients that had received nasal application of mupirocin in the United Kingdom, high-level mupirocin resistant *S. aureus* isolates were not detected.[23] Low-level mupirocin resistance occurred in 1.8% and 5.1% of MSSA and MRSA isolates, respectively, and there was no discernible trend of increasing resistance during the four year study period.

Only one study, in the Netherlands, quantified the occurrence of mupirocin resistance in both *S. aureus* and CoNS after the implementation of universal peri-operative decolonization.[24] Before treatment 21% of all patients carried CoNS with *mupA* mediated high-level resistance and of those patients without such bacteria 37% had *mupA* mediated,

high-level resistance after completing decolonization treatment. No acquisition of high-level mupirocin resistance was detected in *S. aureus* in 939 patients who underwent decolonization therapy. Even though, horizontal gene transfer of the *mupA* gene from *S. epidermidis* to *S. aureus* has been described in vitro and in vivo,[25] no such events were observed in this study. Next to interspecies transfer of *mupA*, clonal dissemination of mupirocin resistant *S. aureus* and CoNS will increase the prevalence of resistance in hospitals.[26] Whether widespread use of chlorhexidine will increase the prevalence of chlorhexidine resistance in staphylococci is unclear,[27] though clonal expansion of MRSA clones expressing chlorhexidine resistance genes has been described.[28] Moreover, the clinical impact of reduced susceptibility to chlorhexidine among *S. aureus* is yet to be determined.[27] We, therefore, restrict our analysis to mupirocin resistance.

Transmission dynamics of mupirocin resistance

We developed a deterministic mathematical model (see online supplementary material for details) to compare the effects of targeted and universal decolonization on the future prevalence of mupirocin resistant *S. aureus* in a hospital setting. High-level mupirocin resistance in *S. aureus* can emerge through clonal spread or through within-host horizontal transmission of *mupA* from CoNS to *S. aureus*.

Setting and model assumptions

For simplicity we used a single ward model, and parameterized the patient admission prevalence of mupirocin-resistant CoNS and *S. aureus*, decolonization rates for *S. aureus* and patient length of stay (Table 1 in the online supplementary material). In the main analysis the admission prevalence is 18.8% for *S. aureus* and 0.08% for mupirocin resistant *S. aureus*. The patient-to-patient transmission rate of *S. aureus* and CoNS was derived from published transmissibility rates of MRSA in hospital settings and quantified as R_A, the single admission reproduction number, defined as the average number of secondary cases generated by one primary case (e.g. a patient colonized with *S. aureus*) during one single hospital admission (see table 1 online appendix).[29,30]

Dynamics of S. aureus and CoNS

Patients are either carriers or non-carriers for *S. aureus* with or without *mupA*. All patients carry CoNS, either *mupA* negative or positive. Only patients carrying *mupA* positive *S. aureus* are not susceptible to acquisition of *mupA* positive CoNS or *S. aureus,* and we ignore any protective effects of colonization with the other species. Application of mupirocin creates selective pressure for acquisition of *mupA* positive staphylococci, which ceases immediately at day five, when application is discontinued. In absence of estimates of the *in-vivo* horizontal gene transfer rates, we assume that these rates are similar for *S. aureus* and CoNS, both *mupA* positive and *mupA* negative. The effects of species-specific horizontal gene transfer rates are explored in sensitivity analyses.

Drivers of mupirocin resistance in S. aureus

Universal decolonization with mupirocin will increase the prevalence of *mupA*-based high-level resistance in CoNS, which increases the opportunities of horizontal gene transfer of *mupA*. Yet, because of the decolonizing effects of mupirocin on *S. aureus* the prevalence of high-level resistance in this species increases only marginally, with no discernible difference in the prevalence of mupirocin resistance in *S. aureus* between targeted and universal decolonization strategies (Figure 1). Increasing the interspecies conjugation rate of *mupA*

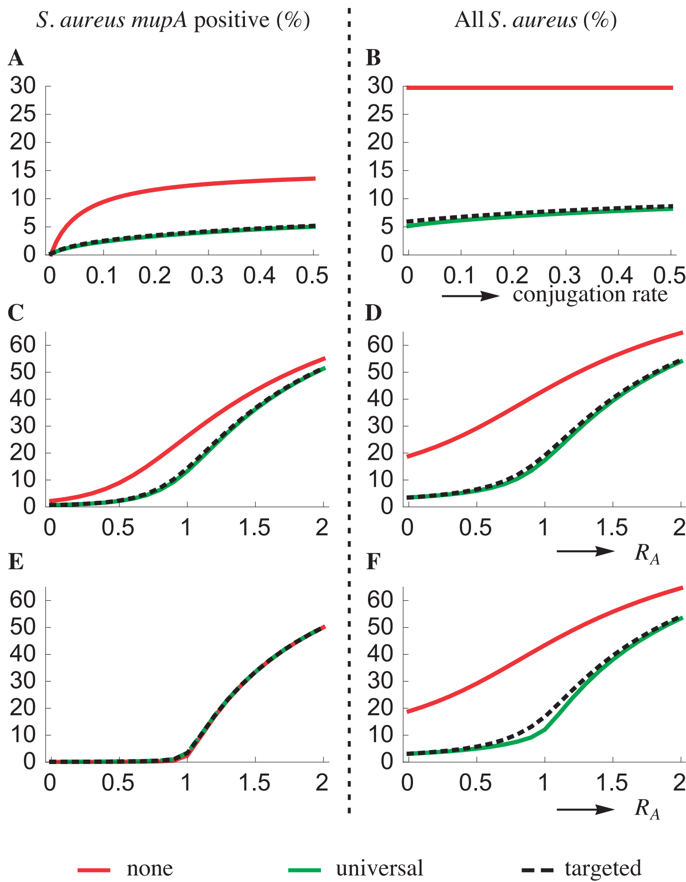


Figure 1. Results of model. Note: Figures A and B: Prevalence of *S. aureus* (*mupA* positive *S. aureus* and all *S. aureus*) with increasing conjugation rates of *mupA* between CoNS and *S. aureus*. Figures C and D: Prevalence of *S. aureus* (*mupA* positive and all *S. aureus*) with increasing R_A. Figures E and F: Prevalence of *S. aureus* (*mupA* positive *S. aureus* and all *S. aureus*) with increasing R_A and no horizontal gene transfer of *mupA* between CoNS and *S. aureus*. Parameters used: Figure A and B: R_A: 0.52; Figures; C and D: conjugation rate: 0.1; Figure E and F: conjugation rate: 0. All figures: percentage of patients colonized on admission: *S. aureus* 18.8%, *mupA* positive *S. aureus* 0.06%, no colonization with *S. aureus* 81.2%, CoNS 79%, CoNS with *mupA* 21%.

between CoNS and *S. aureus* hardly changes these dynamics. Of note, in the absence of decolonization (either targeted or universal) an increased interspecies conjugation rate would, in combination with a high prevalence of patients carrying mupirocin resistant CoNS on admission, increase the rate of mupirocin resistant *S. aureus* significantly, due to the presence of a higher number of mupirocin-susceptible *S. aureus* recipients for *mupA* genes from CoNS (Figure 1).

According to the model the transmission capacity of *S. aureus*, quantified as R_A, is the main driver for an increase of high-level mupirocin resistant *S. aureus* (Figure 1). Without horizontal transfer of *mupA*, there will be no difference in the prevalence of mupirocin resistant *S. aureus* between the two decolonization strategies or no decolonization (Figure 1). Any increase in R_A leads to an increased prevalence of mupirocin resistant *S. aureus*, but this is not influenced by the type of decolonization strategy.

Changing the admission prevalence of mupirocin resistant CoNS and *S. aureus*, as well as of mupirocin sensitive *S. aureus* did not change the results of the model (see figure 3A up to figure 6A, online appendix). In fact, the prevalence of mupirocin resistant *S. aureus* does not differ between decolonization strategies when the admission prevalence of mupirocin resistant *S. aureus* increases. In such a scenario, decolonization will become increasingly less successful in both strategies. And as mupirocin resistant *S. aureus* already contain the *mupA* gene, horizontal gene transfer of *mupA* genes does not occur effectively.

DISCUSSION

Peri-operative decolonization of *S. aureus* carriage is associated with significant health-care gains and cost savings due to prevention of *S. aureus* SSI. Universal decolonization without screening for *S. aureus* carriage is more cost-effective than targeted decolonization based on pre-operative screening. Yet, these benefits should be balanced against the risk of selecting mupirocin resistance in patients not carrying *S. aureus* on admission. The current evidence on the occurrence of high-level mupirocin resistance in *S. aureus* and CoNS, in combination with the results of mathematical modelling, strongly suggest that the increased selection of high-level mupirocin resistance in CoNS does not constitute an important risk for high-level mupirocin resistance in *S. aureus*.

Several assumptions were made in the mathematic model that should be discussed. First, we assumed the transmission capacity of *S. aureus* and CoNS, defined by $R_{A,}$ to be identical, though little is known about transmission capacities of MSSA and CoNS in hospital settings. The R_A-values used were derived from studies quantifying the transmission capacity of MRSA in low-endemic settings.[29,30] Nosocomial transmission of CoNS is rarely studied. In a Swedish ICU 14 of 20 patients were involved in at least one and up to eight probable transmission events.[31] Second, the percentage of patients carrying mupirocin

resistant CoNS and *S. aureus* on admission were based on a setting in which mupirocin had been used in a universal decolonization strategy for two years. Although not studied, this admission prevalence could have been influenced by the universal decolonization strategy, and prevalence might have been lower if less mupirocin had been used as part of a targeted strategy. However, modelling results were not sensitive to the prevalence of mupirocin resistance among CoNS before treatment (online appendix). Third, the horizontal gene transfer rate from CoNS to *S. aureus* was based on a single study, with relatively short follow-up of patients. Yet, the observed prevalence of *mupA* high level resistance in CoNS in that study and the absence of high-level mupirocin resistance in multiple *S. aureus* collections in the Netherlands provides further evidence that horizontal gene transfer does not occur frequently.

Feasibility and cost issues have withheld centers from implementing these measures. The same feasibility and costs issues favor the strategy of universal peri-operative decolonization. Our findings, though partly based on modelling, strongly suggest that the consequent use of mupirocin in those patients not carrying *S. aureus* does not extensively increase the risk of emergence of high-level mupirocin resistance in *S. aureus*. Quantification of the duration of carriage with high-level mupirocin-resistant CoNS and of horizontal gene transfer rates in patients with longer follow-up would allow a more accurate assessment of the ecological safety of universal decolonization with mupirocin in surgical patients.

The impact of universal decolonization regimens with mupirocin in Intensive care units (ICU) to limit transmission and infections caused by MRSA (as performed in Huang et al.[32]), has yet to be determined. However, the dynamics of mupirocin resistance in the ICU setting do not differ substantially from those in surgical patients. Based on the observed low frequencies of within-host horizontal gene transfer of *mupA* from CoNS to *S. aureus*, and the observation that dynamics hardly change with higher horizontal gene transfer rates, it is very likely that cross-transmission rates will also be the most relevant parameter in ICUs. Because of the higher frequency of health-care worker patient contacts in ICUs, repeated introduction of mupirocin resistant *S. aureus* may constitute a risk for the emergence of resistance trough cross-transmission events. Therefore, an observed increase in the prevalence of such bacteria strongly suggests failing infection control procedures.

Even though the beneficial effects of eradicating *S. aureus* carriage before surgery are well established, survey results yielded that only 37%-60% of hospitals in the United States have implemented decolonization strategies for *S. aureus* prior to surgical procedures and that current practices vary widely.[33,34] As both targeted and universal peri-operative decolonization are effective and cost saving it should be a priority for hospitals to implement either one of these strategies. Considering that universal screening for MRSA at the time of hospital admission is already performed in many hospitals, it should be straightforward to also implement testing for MSSA.

FUNDING

This study was performed using departmental funds.

TRANSPARENCY DECLARATIONS

DJH has received a speaker fee from Cepheid Inc.

REFERENCES

1. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis*. 2005;5(12):751-762.
2. Kluytmans JA, Mouton JW, Ijzerman EP, et al. Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. *J Infect Dis*. 1995;171(1):216-219.
3. Kalmeijer MD, van Nieuwland-Bollen E, Bogaers-Hofman D, de Baere GA. Nasal carriage of *Staphylococcus aureus* is a major risk factor for surgical-site infections in orthopedic surgery. *Infect Control Hosp Epidemiol*. 2000;21(5):319-323.
4. Perl TM, Cullen JJ, Wenzel RP, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. *N Engl J Med*. 2002;346(24):1871-1877.
5. van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: A systematic review. *J Antimicrob Chemother*. 2008;61(2):254-261.
6. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med*. 2010;362(1):9-17.
7. Schweizer ML, Chiang HY, Septimus E, et al. Association of a bundled intervention with surgical site infections among patients undergoing cardiac, hip, or knee surgery. *JAMA*. 2015;313(21):2162-2171.
8. Hacek DM, Robb WJ, Paule SM, Kudrna JC, Stamos VP, Peterson LR. *Staphylococcus aureus* nasal decolonization in joint replacement surgery reduces infection. *Clin Orthop Relat Res*. 2008;466(6):1349-1355.
9. Kluytmans JA, Manders MJ, van Bommel E, Verbrugh H. Elimination of nasal carriage of *Staphylococcus aureus* in hemodialysis patients. *Infect Control Hosp Epidemiol*. 1996;17(12):793-797.
10. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and eradication of staphylococcus aureus carriage. *PLoS One*. 2011;6(5):e14815.
11. Andriesse GI, van Rijen M, Bogaers D, Bergmans AM, Kluytmans JA. Comparison of two PCR-based methods and conventional culture for the detection of nasal carriage of *Staphylococcus aureus* in pre-operative patients. *Eur J Clin Microbiol Infect Dis*. 2009;28(10):1223-1226.
12. Choudhurry RS, Melles DC, Eadie K, et al. Direct detection of human *Staphylococcus aureus* carriage in the nose using the lightcycler staphylococcus kit. *J Microbiol Methods*. 2006;65(2):354-356.
13. Luzar MA, Coles GA, Faller B, et al. *Staphylococcus aureus* nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. *N Engl J Med*. 1990;322(8):505-509.
14. White A. Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. *Antimicrob Agents Chemother (Bethesda)*. 1963;161:667-670.
15. Matheson A, Christie P, Stari T, et al. Nasal swab screening for methicillin-resistant *Staphylococcus aureus*--how well does it perform? A cross-sectional study. *Infect Control Hosp Epidemiol*. 2012;33(8):803-808.

16. Vivoni AM, Santos KR, de-Oliveira MP, et al. Mupirocin for controlling methicillin-resistant *Staphylococcus aureus*: Lessons from a decade of use at a university hospital. *Infect Control Hosp Epidemiol*. 2005;26(7):662-667.
17. Talon D, Marion C, Thouverez M, Bertrand X. Mupirocin resistance is not an inevitable consequence of mupirocin use. *J Hosp Infect*. 2011;79(4):366-367.
18. Lee AS, Macedo-Vinas M, Francois P, et al. Trends in mupirocin resistance in meticillin-resistant *Staphylococcus aureus* and mupirocin consumption at a tertiary care hospital. *J Hosp Infect*. 2011;77(4):360-362.
19. Torvaldsen S, Roberts C, Riley TV. The continuing evolution of methicillin-resistant staphylococcus aureus in Western Australia. *Infect Control Hosp Epidemiol*. 1999;20(2):133-135.
20. Riley TV, Carson CF, Bowman RA, et al. Mupirocin-resistant methicillin-resistant *Staphylococcus aureus* in Western Australia. *Med J Aust*. 1994;161(6):397-398.
21. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. *J Hosp Infect*. 2013;85(4):249-256.
22. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant *Staphylococcus aureus* carriage after decolonization therapy: A case-control study. *Clin Infect Dis*. 2011;52(12):1422-1430.
23. Fawley WN, Parnell P, Hall J, Wilcox MH. Surveillance for mupirocin resistance following introduction of routine peri-operative prophylaxis with nasal mupirocin. *J Hosp Infect*. 2006;62(3):327-332.
24. Hetem DJ, Vogely HC, Severs TT, Troelstra A, Kusters JG, Bonten MJ. Acquisition of high-level mupirocin resistance in CoNS following nasal decolonization with mupirocin. *J Antimicrob Chemother*. 2015;70(4):1182-1184.
25. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from staphylococcus epidermidis to methicillin-resistant *Staphylococcus aureus* associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother*. 2005;56(6):1166-1168.
26. Petinaki E, Spiliopoulou I, Kontos F, et al. Clonal dissemination of mupirocin-resistant staphylococci in greek hospitals. *J Antimicrob Chemother*. 2004;53(1):105-108.
27. Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: Is it increasing and does it matter? *J Antimicrob Chemother*. 2012;67(11):2547-2559.
28. Sekiguchi J, Hama T, Fujino T, et al. Detection of the antiseptic- and disinfectant-resistance genes qacA, qacB, and qacC in methicillin-resistant *Staphylococcus aureus* isolated in a Tokyo hospital. *Jpn J Infect Dis*. 2004;57(6):288-291.
29. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis*. 2013;19(11):1797-1802.
30. McBryde ES, Pettitt AN, McElwain DL. A stochastic mathematical model of methicillin resistant *Staphylococcus aureus* transmission in an intensive care unit: Predicting the impact of interventions. *J Theor Biol*. 2007;245(3):470-481.
31. Agvald-Ohman C, Lund B, Edlund C. Multiresistant coagulase-negative staphylococci disseminate frequently between intubated patients in a multidisciplinary intensive care unit. *Crit Care*. 2004;8(1):R42-7.
32. Huang SS, Septimus E, Kleinman K, et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med*. 2013;368(24):2255-2265.
33. Kline S, Highness M, Herwaldt LA, Perl TM. Variable screening and decolonization protocols for *Staphylococcus aureus* carriage prior to surgical procedures. *Infect Control Hosp Epidemiol*. 2014;35(7):880-882.
34. Diekema D, Johannsson B, Herwaldt L, et al. Current practice in *Staphylococcus aureus* screening and decolonization. *Infect Control Hosp Epidemiol*. 2011;32(10):1042-1044.

SUPPLEMENTARY APPENDIX

Mathematical model

We have developed a deterministic mathematical model (see Figure 1A, Figure 2A and Equations 1A) to analyze the effect of universal and selective use of mupirocin to decolonize carriers of *Staphylococcus aureus*.

We consider a single ward with 60 beds, which we assume are always occupied. As the ward size is relatively large and because we assume that the prevalence of mupirocin resistance at admission is non-negligible, results of a deterministic model and a stochastic model will be similar. We have checked this for several parameter values. For simplicity and clarity we only present the results of the deterministic model.

We assume all patients carry coagulase-negative staphylococci (CoNS), either susceptible to mupirocin ($CoNS^-$) or resistant to mupirocin ($CoNS^+$). Patients may not carry *S. aureus* (SA), may be colonized with mupirocin-susceptible *S. aureus* (SA^-) or may be colonized with mupirocin-resistant *S. aureus* (SA^+). This leads to six different colonization states of a patient. We label these six categories from A to F (see Figure 1A).

nine

We assume that the colonization status of a patient with respect to CoNS and *S. aureus* has no influence on the length of stay, which we assume to be exponentially distributed with a mean of 12.2 days.[1] We assume that CoNS and SA can be transmitted in the ward via cross-transmission and we assume, in absence of information in the literature, that the single admission reproduction number for CoNS and *S. aureus* is the same as for methicillin-resistant *S. aureus* (MRSA), i.e., R_A=0.52.[2,3] As a worst-case scenario for mupirocin use, we assume that patients already colonized with SA^- are equally likely to acquire SA^+ as patients who are not colonized with *S. aureus*, i.e., we ignore the colonization resistance. A patient colonized with $CoNS^+$ and SA^- may also acquire SA^+ due to conjugation, and a patient colonized with $CoNS^-$ and SA^+ may acquire $CoNS^+$ due to conjugation. In absence of good estimates of the *in vivo* conjugation rates, we assume that both conjugation rates are the same and we vary the conjugation rates in our analysis.

We now add the use of mupirocin to our model. We consider two main scenarios, 1) universal application of mupirocin, i.e., all patients in the ward are treated with mupirocin during the first five days after admission, and 2) selective application of mupirocin; we assume that at admission all patients are screened for carriage with *S. aureus*, that culture results are immediately available and that only patients colonized with *S. aureus* receive mupirocin for the first five days. This resembles the optimal scenario for selective application, i.e., there is no delay in detection and no misclassification of carriage. More explicitly, we assume that mupirocin is given for on average five days with a standard deviation of one day (Gamma distributed with shape parameter 16 and scale parameter 5/16).

If a patient receives mupirocin, other events can occur as well. Most importantly, patients colonized with SA^- lose their colonization; 94% of the patients (424 of 453 carriers) lost colonization after treatment.[4] This results in a decolonization rate of 0.6 per

day. However, application of mupirocin may also select for $CoNS^+$ or $CoNS^-$ may acquire resistance, we assume this occurs in 37% of the patients receiving a five day mupirocin course. This corresponds to a rate of 7.4/100 patient days at risk.[5] Moreover, mupirocin use may select for resistance in *S. aureus* as well. This occurs in 1% of the treated patients,[4] which corresponds to a rate of 0.002/day.

Importantly, we assume that there is no delayed effect of mupirocin treatment, i.e., two patients with the same colonization status have identical transition rates, irrespective of mupirocin use in the past.

To ensure a Gamma-distribution for the duration of mupirocin use, we subdivided each category representing a colonization state into multiple states $0, ..., n$, where $n = 16$. We define $X = \sum_{j=0}^{n} X_j$ for all categories, i.e., for $X \in \{A, B, C, D, E, F, G\}$. Our assumptions and definitions lead to the following set of differential equations for $1 \leq i < n = 16$ where $\varepsilon = 1$ if there is universal application of mupirocin and $\varepsilon = 0$ if there is selective screening. The parameters and their default values are defined in Table 1A.

Table 1A. Parameters mupirocin resistance model.

Parameter	Symbol	Value	Source and Remarks
Single admission reproduction number of *S. aureus* / CoNS	R_A	0.52	Hetem et al. and McBryde et al.[2,3]
Mean length of stay	$1/\mu$	12.2 days	Bode et al.[1]
Transmission parameter	$\beta = R_A \mu$	*0.043*	
Acquisition rate of mupirocin resistance in mupirocin susceptible CoNS during decolonization	σ	0.074/day	Hetem et al.[5]
Acquisition rate of mupirocin resistance in mupirocin susceptible *S. aureus* during decolonization	m	0.002/day	Ammerlaan et al.[4]
Decolonization rate of *S. aureus* after 1 week	γ	0.6/day	Ammerlaan et al.[4]
Conjugation rate	φ		No *in vivo* data
Dummy variable to ensure a Gamma-distributed duration of mupirocin use	ω	16/5	
Percentage of patients colonized at admission*			
S. aureus, mupirocin susceptible		18.8%	Bode et al.[1]
S. aureus, mupirocin resistant		0.06%	Hetem et al.[5]
CoNS, mupirocin susceptible		79%	Based on assumption that everybody is colonized with CoNS nasally. Hetem et al.[5]
CoNS, mupirocin resistant		21%	Hetem et al.[5]

* The admission prevalence in categories A, B, C, D, E, F, represented by the parameters p_A, p_B, p_C, p_D, p_E, p_F, are calculated assuming that colonization with CoNS and colonization with *S. aureus* are independent of each other.

nine

Equations 1A

$$\frac{dA_0}{dt} = \mu\epsilon p_A + \gamma C_0 - (\mu + \sigma_A + \beta^{CNS}(B + D + F) + \beta^{SA+}(E + F) + \beta^{SA-}(C + D) + \omega)A_0$$

$$\frac{dA_i}{dt} = \gamma C_i + \omega A_{i-1} - (\mu + \sigma_A + \beta^{CNS}(B + D + F) + \beta^{SA+}(E + F) + \beta^{SA-}(C + D) + \omega)A_i$$

$$\frac{dA_n}{dt} = \mu(1 - \epsilon)p_A + \gamma_n C_n + \omega A_{n-1} - \big(\mu + \beta^{CNS}(B + D + F) + \beta^{SA+}(E + F) + \beta^{SA-}(C + D)\big)A_n$$

$$\frac{dB_0}{dt} = (\beta^{CNS}(B + D + F) + \sigma_A)A_0 + \mu\epsilon p_B + \gamma D_0 - (\mu + \beta^{SA-}(C + D) + \beta^{SA+}(E + F) + \omega)B_0$$

$$\frac{dB_i}{dt} = (\beta^{CNS}(B + D + F) + \sigma_A)A_i + \omega B_{i-1} + \gamma D_i - (\mu + \beta^{SA-}(C + D) + \beta^{SA+}(E + F) + \omega)B_i$$

$$\frac{dB_n}{dt} = \beta^{CNS}(B + D + F)A_n + \mu(1 - \epsilon)p_B + \omega B_{n-1} - \big(\mu + \beta^{SA+}(E + F) + \beta^{SA-}(C + D)\big)B_n$$

$$\frac{dC_0}{dt} = \mu p_C + \beta^{SA-}(C + D)A_0 - (\mu + \gamma + \beta^{CNS}(B + D + F) + \beta^{SA+}(E + F) + \sigma_C + m + \omega)C_0$$

$$\frac{dC_i}{dt} = \beta^{SA-}(C + D)A_i + \omega C_{i-1} - (\mu + \beta^{SA+}(E + F) + \beta^{CNS}(B + D + F) + \gamma + \sigma_C + m + \omega)C_i$$

$$\frac{dC_n}{dt} = \beta^{SA-}(C + D)A_n + \omega C_{n-1} - (\mu + \beta^{SA+}(E + F) + \beta^{CNS}(B + D + F) + \gamma_n + m_n)C_n$$

$$\frac{dD_0}{dt} = \beta^{SA-}(C + D)B_0 + (\beta^{CNS}(B + D + F) + \sigma_C)C_0 + \mu p_D - (\mu + \beta^{SA+}(E + F) + \omega + \varphi_D + m + \gamma)D_0$$

$$\frac{dD_i}{dt} = \beta^{SA-}(C + D)B_i + (\beta^{CNS}(B + D + F) + \sigma_C)C_i + \omega D_{i-1} - (\mu + \beta^{SA+}(E + F) + \omega + \varphi_D + m + \gamma)D_i$$

$$\frac{dD_n}{dt} = \beta^{SA-}(C + D)B_n + \big(\beta^{CNS}(B + D + F)\big)C_i + \omega D_{n-1} - (\mu + \beta^{SA+}(E + F) + \varphi_D + m_n)D_n$$

$$\frac{dE_0}{dt} = \mu p_E + \beta^{SA+}(E + F)(A_0 + C_0) + mC_0 - (\mu + \beta^{CNS}(B + D + F) + \sigma_E + \varphi_E + \omega)E_0$$

$$\frac{dE_i}{dt} = \beta^{SA+}(E + F)(A_i + C_i) + mC_i + \omega E_{i-1} - (\mu + \beta^{CNS}(B + D + F) + \sigma_E + \varphi_E + \omega)E_i$$

$$\frac{dE_n}{dt} = \beta^{SA+}(E + F)(A_n + C_n) + m_n C_n + \omega E_{n-1} - (\mu + \beta^{CNS}(B + D + F) + \varphi_E)E_n$$

$$\frac{dF_0}{dt} = \beta^{SA+}(E + F)(B_0 + D_0) + (\beta^{CNS}(B + D + F) + \sigma_E + \varphi_E)E_0 + (\varphi_D + m)D_0 + \mu p_F - (\mu + \omega)F_0$$

$$\frac{dF_i}{dt} = \beta^{SA+}(E + F)(B_i + D_i) + (\beta^{CNS}(B + D + F) + \sigma_E + \varphi_E)E_i + \omega F_{i-1} + (\varphi_D + m)D_i - (\mu + \omega)F_i$$

$$\frac{dF_n}{dt} = \beta^{SA+}(E + F)(B_n + D_n) + (\beta^{CNS}(B + D + F) + \varphi_E)E_n + \omega F_{n-1} + (\varphi_D + m_n)D_n - \mu F_n$$

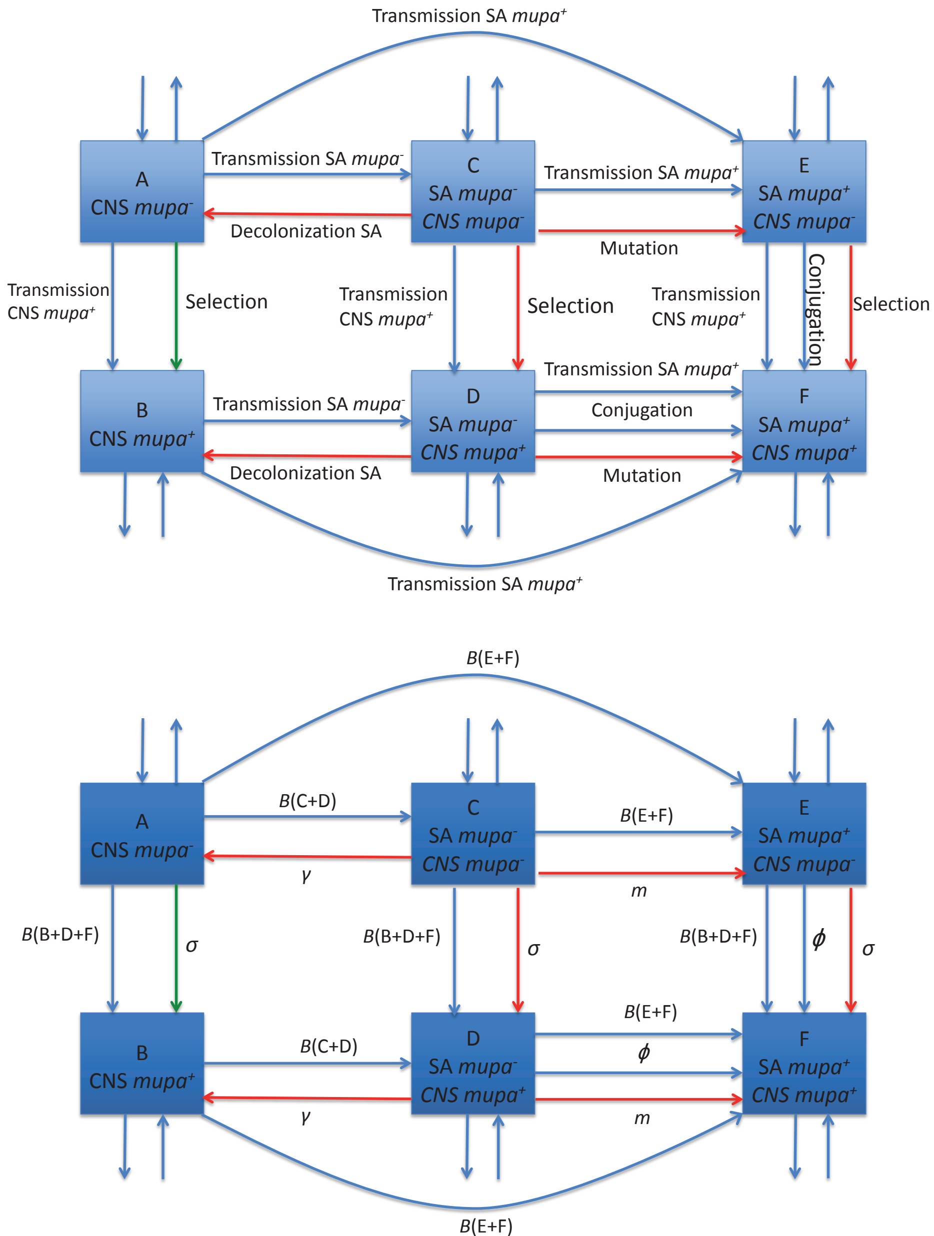


Figure 1A and 2A. Schematic representation of the model. In Figure1 A we have described the transitions, in Figure 2A we show the per capita rate. Blue arrows correspond to transitions that are present irrespective of mupirocin use. The red arrows are present for patients who were colonized with SA on admission during their first 5 days. The green arrow is only present in case of universal application of mupirocin.

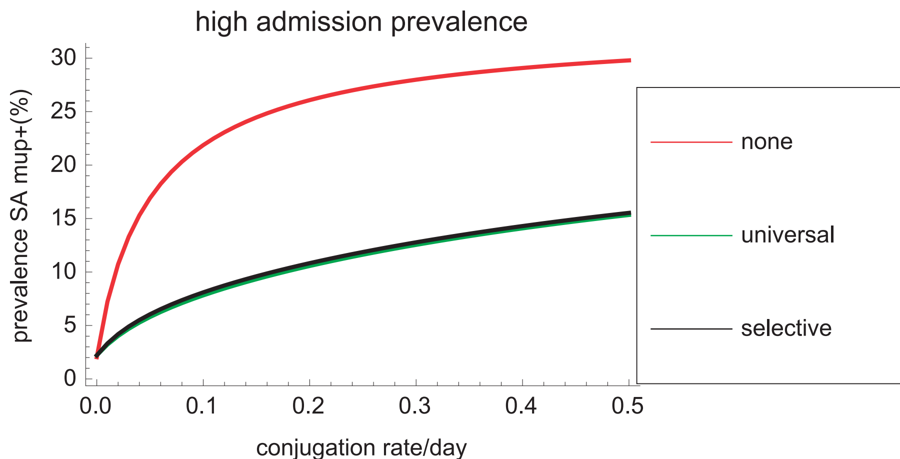


Figure 3A. Prevalence of *mupA* positive *S. aureus* with increasing conjugation rate with high prevalence of mupirocin resistant *S. aureus* and CoNS on admission
Note: Percentage of patients colonized on admission : S. *aureus* 30%, *mupA* positive *S. aureus* 1%, no colonization with *S. aureus* 69%, CoNS 50%, CoNS with *mupA* 50%.

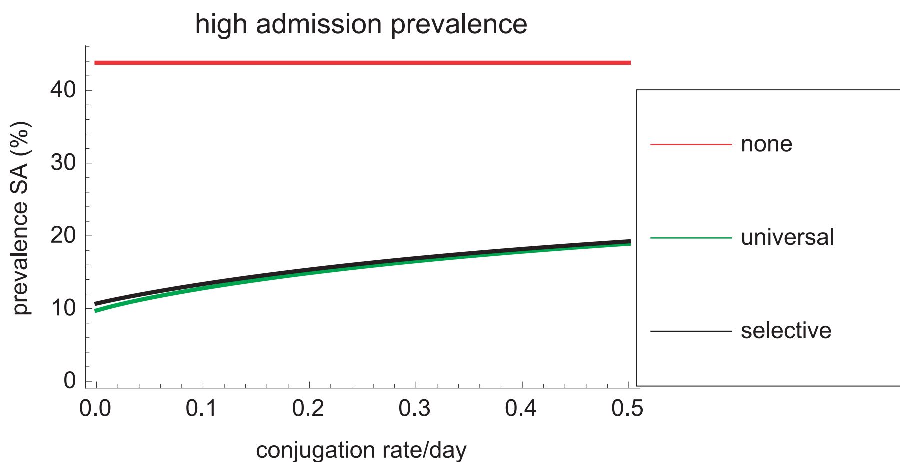


Figure 4A. Prevalence of *S. aureus* with increasing conjugation rate with high prevalence of mupirocin resistant *S. aureus* and CoNS on admission
Note: Percentage of patients colonized on admission : *S. aureus* 30%, *mupA* positive *S. aureus* 1%, no colonization with *S. aureus* 69%, CoNS 50%, CoNS with *mupA* 50%.

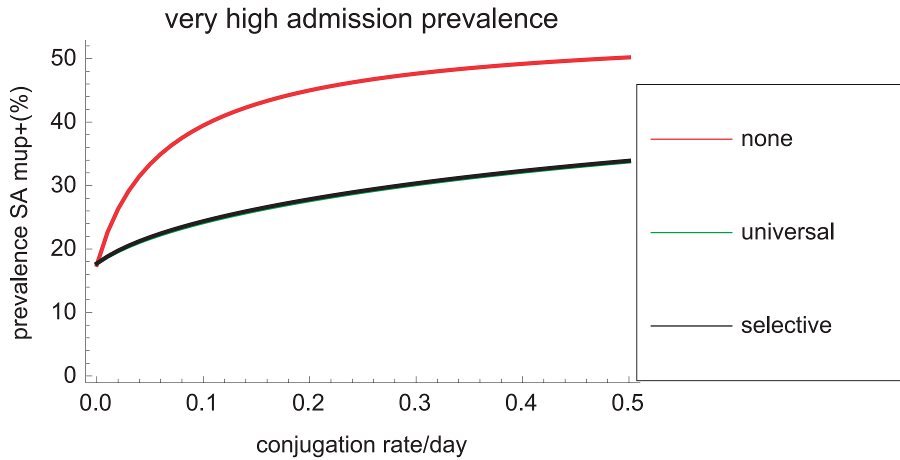


Figure 5A. Prevalence of *mupA* positive *S. aureus* with increasing conjugation rate with very high prevalence of mupirocin resistant *S. aureus* and CoNS on admission
Note: Percentage of patients colonized on admission : *S. aureus* 50%, *mupA* positive *S. aureus* 10%, no colonization with *S. aureus* 40%, CoNS 50%, CoNS with *mupA* 50%.

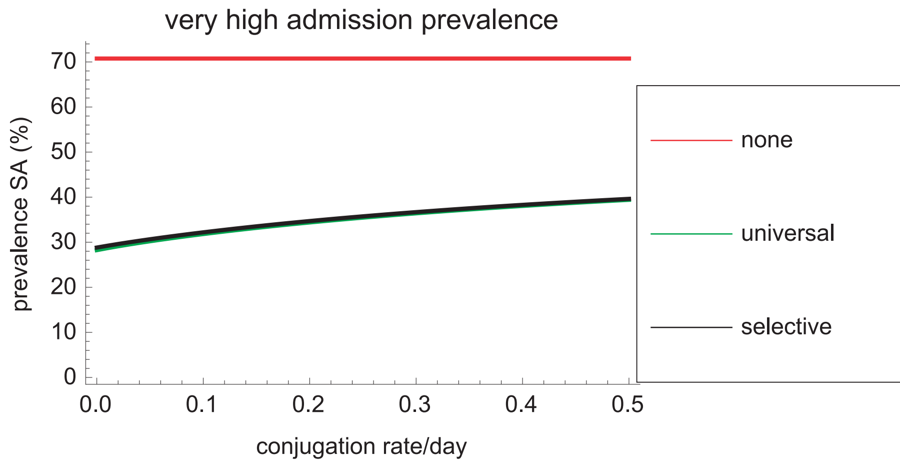


Figure 6A. Prevalence of *S. aureus* with increasing conjugation rate with high prevalence of mupirocin resistant *S. aureus* and CoNS on admission
Note: Percentage of patients colonized on admission : *S. aureus* 50%, *mupA* positive *S. aureus* 10%, no colonization with *S. aureus* 40%, CoNS 50%, CoNS with *mupA* 50%.

REFERENCES

1. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of staphylococcus aureus. *N Engl J Med*. 2010;362(1):9-17.
2. McBryde ES, Pettitt AN, McElwain DL. A stochastic mathematical model of methicillin resistant staphylococcus aureus transmission in an intensive care unit: Predicting the impact of interventions. *J Theor Biol*. 2007;245(3):470-481.
3. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant staphylococcus aureus. *Emerg Infect Dis*. 2013;19(11):1797-1802.
4. Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ. Eradication of methicillin-resistant staphylococcus aureus carriage: A systematic review. *Clin Infect Dis*. 2009;48(7):922-930.
5. Hetem DJ, Vogely HC, Severs TT, Troelstra A, Kusters JG, Bonten MJ. Acquisition of high-level mupirocin resistance in CoNS following nasal decolonization with mupirocin. *J Antimicrob Chemother*. 2015;70(4):1182-1184.

part IV

MANAGEMENT OF INTRAVASCULAR CATHETERS COLONIZED WITH *STAPHYLOCOCCUS AUREUS*

ten

PREVENTING *STAPHYLOCOCCUS AUREUS* BACTEREMIA AND SEPSIS IN PATIENTS WITH *STAPHYLOCOCCUS AUREUS* COLONIZATION OF INTRAVASCULAR CATHETERS: A RETROSPECTIVE MULTICENTER STUDY AND META-ANALYSIS

David J. Hetem, Suzanne C. de Ruiter, Anton G.M. Buiting, Jan A.J.W. Kluytmans, Steven F. Thijsen, Bart J.M. Vlaminckx, Robert G.F. Wintermans, Marc J.M. Bonten, Miquel B. Ekkelenkamp

Medicine, 2011

ABSTRACT

Two previous studies in tertiary care hospitals identified *Staphylococcus aureus* colonization of intravascular (IV) catheters as a strong predictor of subsequent *S. aureus* bacteremia (SAB), even in the absence of clinical signs of systemic infection. Bacteremia was effectively prevented by timely antibiotic therapy. We conducted this study to corroborate the validity of these findings in non-university hospitals.

Using the laboratory information management systems of the clinical microbiology departments in 6 Dutch hospitals, we identified patients who had IV catheters from which *S. aureus* was cultured between January 1, 2003, and December 31, 2008. Patients with demonstrated SAB between 7 days before catheter removal and 24 hours after catheter removal were excluded. We extracted clinical and demographic patient data from the patients' medical records. The primary risk factor was initiation of anti-staphylococcal antibiotic therapy within 24 hours, and the primary endpoint was SAB 924 hours after IV catheter removal. Subsequently, we performed a systematic review and meta-analysis of all observational studies evaluating the effect of antibiotic therapy for *S. aureus* IV catheter tip colonization.

In the current study, 18 of the 192 included patients developed subsequent SAB, which was associated with not receiving antibiotic therapy within 24 hours (odds ratio [OR], 4.2; 95% confidence interval [CI], 1.1-15.6) and with documented exit-site infection (OR, 3.3; 95% CI, 1.2-9.3). When we combined these results with results of a previous study in a university hospital, a third risk factor was also associated with subsequent SAB, namely corticosteroid therapy (OR, 2.9; 95% CI, 1.3-6.3). We identified 3 other studies, in addition to the present study, in a systematic review. In the meta-analysis of these studies, antibiotic therapy yielded an absolute risk reduction of 13.6% for subsequent SAB. The number needed to treat to prevent 1 episode of SAB was 7.4. We conclude that early initiation of antibiotic therapy for IV catheters colonized with *S. aureus* prevents subsequent SAB.

Abbreviations: CI = confidence interval, CVC = central venous catheter, IDSA = Infectious Diseases Society of America, IV = intravascular, MRSA = methicillin-resistant Staphylococcus aureus, OR = odds ratio, q4h = every 4 hours, q8h = every 8 hours, q12h = every 12 hours, SAB = Staphylococcus aureus bacteremia.

INTRODUCTION

Catheter-associated *Staphylococcus aureus* bacteremia (SAB) is a severe health care-associated infection that may result in septic thrombosis, peripheral abscesses, endocarditis, and death.[1,2] Yet, catheter colonization frequently occurs without clinical signs of infection, and often without evidence of concomitant SAB. Whether these patients, with growth of *S. aureus* on catheter tips and without symptoms of infection, should receive antimicrobial treatment for SAB has not been firmly established, although many physicians probably decide to treat such patients with antibiotics, regardless of the patients' clinical condition.

Recently, 2 retrospective studies[3,4] identified *S. aureus* colonization of intravascular (IV) catheters as a risk factor for subsequent SAB, even in patients who did not exhibit signs of local or systemic infection at the time of catheter removal; furthermore, antibiotic therapy initiated within 24 hours after catheter removal was associated with a lower risk of subsequent SAB. Based on these findings, the Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and management of IV catheter-related infection now recommend 5-7 days of antibiotic treatment for patients with demonstrated *S. aureus* colonization of central venous catheters (CVCs), although they do not specify an antibiotic of choice, or a preference for a mode of administration.[5] Yet, all evidence for this recommendation originates from 2 retrospective studies with 176 patients in total and performed in 2 tertiary care hospitals. It is unknown to what extent the findings of these studies were influenced by the specific patient population of each tertiary care center.

We, therefore, quantified the risks of catheter tip colonization with *S. aureus* and subsequent development of SAB, and the effects of antimicrobial therapy thereon, in a less severely ill population of patients treated in 6 non-university hospitals.

PATIENTS AND METHODS

Patients and Patient Data

Patients were included from 6 Dutch non-university hospitals: the Diakonessenhuis Utrecht, the St. Antonius Hospitals in Nieuwegein and Utrecht (2 locations), the Amphia Hospital in Breda (2 locations), the St. Elisabeth Hospital in Tilburg, the Twee Steden Hospital in Tilburg, and the Franciscus Hospital in Roosendaal. We identified patients with IV catheters colonized with *S. aureus* during the period January 2003 to December 2008 using the microbiology laboratory information management systems of these hospitals' microbiologic departments. We searched the same databases for blood cultures taken between 7 days before IV catheter removal through 6 months after removal. Patients with SAB from 7 days before through 24 hours after removal of the IV catheter were excluded. We reviewed patient medical records to retrieve demographic, clinical, and laboratory data. The following potential risk factors were extracted: age, sex, location and type of the IV catheter, duration of catheterization, underlying disease and co-morbid conditions,

mechanical ventilation, duration of hospital stay, signs of local exit-site infection, symptoms of systemic inflammatory response syndrome (SIRS), immunosuppressive therapy, use of antibiotic therapy including type of antibiotic used, and duration and day of initiation of antibiotics. The follow-up period was 6 months.

Definitions

A subsequent bacteremic complication was defined as 1) SAB >24 hours after removal of the IV catheter, and 2) an identical susceptibility pattern in the blood culture isolate and the isolate from the IV catheter tip. Antibiotic treatment was defined as initiation within 24 hours of antibiotic therapy (oral or intravenous) to which the cultured strain was susceptible, and continuation of this antibiotic therapy for a minimum of 3 days. Exit-site infection was defined as erythema, swelling, purulence, and/or tenderness at the IV catheter exit site.

Culture

Catheter tips were processed as described by Maki et al.[6] A catheter tip culture yielding more than 15 colony-forming units of *S. aureus* was considered positive. Blood cultures were incubated for at least 5 days in all participating hospitals. Identification and susceptibility testing were performed using an automated system and software; the identification was confirmed by testing free and bound coagulase.

Statistical Analysis and Literature Search

We analyzed data using SPSS for Windows (v. 15.0). Nominal variables were analyzed by chi-square test and the Fisher exact test where appropriate; continuous variables were analyzed by the Mann-Whitney U test. Significance was assessed 2-sided for all variables, applying a cutoff value of $p < 0.05$. Risk factors with a p value < 0.1 in the univariate analysis were subsequently analyzed by multivariate logistic regression analysis (backward conditional) to calculate odds ratios (OR) and 95% confidence intervals (95% CI).

We conducted a literature search of the MEDLINE database (National Library of Medicine, Bethesda, MD) to identify publications on the relation between *S. aureus* catheter tip colonization, antibiotic therapy, and SAB that were published up to May 2010. Keywords used for the search were ''*Staphylococcus aureus*'' AND ''catheter.'' We reviewed the reference lists of identified publications until we could identify no more new publications. The meta-analysis was carried out using Review Manager (v. 5.0.24, Cochrane Collaboration, Oxford, UK). We performed a Mantel-Haenszel analysis using the fixed effects model to calculate the pooled OR and 95% CIs. We used I^2 - statistics to assess heterogeneity.

RESULTS

We identified 450 patients with an IV catheter colonized with *S. aureus* during the 6-year study period. Of these, 256 (57%) were excluded because they had positive blood cultures

with *S. aureus* drawn between 7 days prior until 24 hours after removal of the catheter, and 2 patients were excluded because we could not determine from their medical records whether antibiotic treatment had been started within 24 hours of catheter extraction (neither patient had subsequent SAB). A total of 192 patients were included (Table 1): 20 (10%) with tunneled CVCs, 62 (32%) with subclavian CVCs, 61 (32%) with jugular CVCs, 30 (16%) with femoral CVCs, 12 (6%) with arterial catheters, 5 (3%) with umbilical CVCs, and 2 (1%) with peripherally inserted central catheters. The insertion site of 20 (10%) CVCs could not be retrieved. The median duration of catheter insertion was 7 days. Four cultures (2%) yielded methicillin-resistant *S. aureus* (MRSA).

Seventy-four patients received antibiotic therapy active against the cultured *S. aureus* within 24 hours: 37 patients were treated with specific anti-staphylococcal therapy (flucloxacillin, cefazolin, clindamycin, or vancomycin), and another 37 patients received empiric therapy with broad-spectrum antibiotics. In 16 of the latter patients, the therapy was changed to narrow spectrum anti-staphylococcal therapy (flucloxacillin) during the course of treatment.

Eighteen patients developed subsequent SAB an average of 10.7 days (range, 2-65 d) after removal of the catheter: 3 of 74 patients (4%) who received antibiotic therapy within 24 hours compared to 15 of 118 patients (13%) who did not (OR in multivariate analysis for not receiving antibiotic therapy, 4.2; 95% CI, 1.1-15.6) (see Table 1). The only other significant risk factor for subsequent SAB was an exit-site infection at time of catheter removal (OR, 3.34, 95% CI, 1.19-9.34). In 1 patient the drain colonization and subsequent bacteremia were caused by MRSA. In the multivariate logistic regression, signs of an exit-site infection and not receiving antibiotic therapy within 24 hours both gained significance, probably because these 2 factors were inversely correlated (OR, 0.5), as shown in Table 2. Antibiotic therapy was more frequently prescribed in patients with fever than in those without fever (45% vs. 19%, respectively; OR, 2.39; 95% CI, 1.52-3.77) and in patients with documented exit-site infection compared with those without (39% vs. 24%, respectively; OR, 1.65; 95% CI, 1.07-2.54). Three of the 18 patients with subsequent SAB had negative blood cultures around the time of catheter extraction; in the other 15 patients, blood cultures were not drawn until they developed signs of sepsis.

We subsequently added the patient data from our previous study,[3] in which the same methods and definitions were used for 99 patients in a university center (Table 3). In this combined cohort, incidences of SAB were 4% and 16%, respectively, for patients receiving (n = 124) and not receiving (n = 167) antibiotic therapy within 24 hours. These figures correspond to a relative risk of 0.25 (95% CI, 0.10-0.63) for developing subsequent SAB when receiving antibiotic therapy within 24 hours, and a number needed to treat of 8. Multivariate analysis in this larger combined cohort identified 3 risk factors for subsequent SAB: not receiving antibiotic therapy within 24 hours (OR, 5.4; 95% CI, 2.0-15.1), documented exit-site infection (OR, 3.3; 95% CI, 1.5-7.4), and corticosteroid therapy (OR, 2.9; 95% CI, 1.3-6.6).

Table 1. Demographical data, potential risk factors and outcome in patients with intravenous catheter tips colonized with *Staphylococcus aureus*

Variable	Patients without subsequent SAB (n=174)	Patients with subsequent SAB after 48h. (n=18)
Age, mean years	59	63
Male sex	106 (61%)	9 (50%)
Underlying conditions:		
Underlying malignancy	34 (19%)	5 (28%)
Hematological malignancy	9 (5%)	1 (6%)
Diabetes mellitus	30 (17%)	4 (22%)
Hemodialysis	29 (17%)	6 (33%)
Mechanical ventilation	29 (17%)	2 (11%)
COPD	23 (13%)	2 (11%)
Type of catheter:		
Tunneled catheter	17 (10%)	3 (17%)
Catheter insertion site:		
Jugular catheter	55 (32%)	6 (33%)
Subclavian catheter	57 (33%)	5 (28%)
Femoral catheter	24 (14%)	6 (33%)
Umbilical catheter	5 (3%)	0
Arterial catheter	11 (6%)	1 (6%)
Peripherally inserted central catheter	2 (1%)	0
CVC unknown insertion site	20 (11%)	0
Duration of catheterization, median days	7	7
Use of IV-catheter		
Total parental nutrition	40 (23%)	6 (33%)
Inotropic therapy	44 (25%)	4 (22%)
Chemotherapy	10 (6%)	1 (6%)
Fever	47 (27%)	8 (44%)
Documented exit-site infection	48 (28%)	9 (50%)
Immunosuppressive therapy (all)	46 (26%)	7 (39%)
Systemic corticosteroids therapy	34 (19%)	5 (28%)
No antibiotic therapy within 24 hours	103 (59%)	15 (83%)
Initial antibiotic therapy		
β-lactam antibiotics*	62 (36%)	3 (17%)
Vancomycin	3 (2%)	0
Other antibiotics†	7 (4%)	0
Duration of antibiotic therapy, median days	9	17
Oral antibiotics	6 (3%)	1 (5%)

SAB: *Staphylococcus aureus* bacteremia; OR: odds ratio; 95% CI: 95% confidence interval; COPD: chronic obstructive pulmonary disease; CVC: central venous catheter. *β-lactam antibiotics used: flucloxacillin 30, amoxicillin+clavulanic acid 15, cefuroxime 13, ceftriaxone 3, cefazolin 2, ceftazidime 1, cefotaxime 1. †Other

p-value, univariate analysis	OR, univariate analysis (95%CI)	p-value, multivariate analysis	OR, multivariate analysis (95%CI)
0.50			
0.40			
0.42			
0.95			
0.62			
0.086	2.4 (0.86-7.1)	0.30	1.9 (0.55-6.8)
0.54			
0.79			
0.36			
0.84			
0.44			
0.060	2.7 (0.93-7.9)	0.50	1.4 (0.48-4.4)
1.00			
0.80			
1.00			
0.85			
0.45			
0.62			
0.91			
0.12			
0.048	2.6 (0.99-7.1)	0.022	3.3 (1.2-9.3)
0.26			
0.36			
0.045	3.4 (0.96-12.3)	0.034	4.2 (1.1-15.6)

antibiotics used: ciprofloxacin 3, clindamycin 2, co-trimoxazole 2. One patient received combination therapy of amoxicillin+clavulanic acid with ciprofloxacin.

ten

Table 2. Fever and exit-site infection in relation with antibiotic therapy

Variable	Antibiotic therapy within 24 hours (n=74)	No antibiotic therapy within 24 hours (n=118)	p-value
Fever	33/74 (45%)	22/118 (19%)	0.000
Documented exit-site infection	29/74 (39%)	28/118 (24%)	0.022

In the literature search we identified 3 retrospective cohort studies apart from the current study, for a total of 426 patients with *S. aureus* IV catheter tip colonization.[3,4,7] All 4 studies used the culture method and cutoff values described by Maki et al[6] (>15 colony-forming units). Meta-analysis of these studies yielded a pooled OR of 5.8 (95% CI, 2.6-13.2) for subsequent SAB when antibiotic therapy was not initiated (Figure 1). Prompt initiation of antibiotic treatment led to an absolute risk reduction of 13.6%, which corresponds to a number needed to treat of 7.4 patients to prevent 1 case of subsequent SAB. There was no heterogeneity between the studies (I^2 = 0%).

DISCUSSION

In the current study, *S. aureus* colonization of IV catheters was complicated by subsequent SAB (>24 h after catheter removal) in 18 of 192 patients without manifest SAB at the time of catheter removal; this complication could be prevented by prompt initiation of antibiotic therapy.

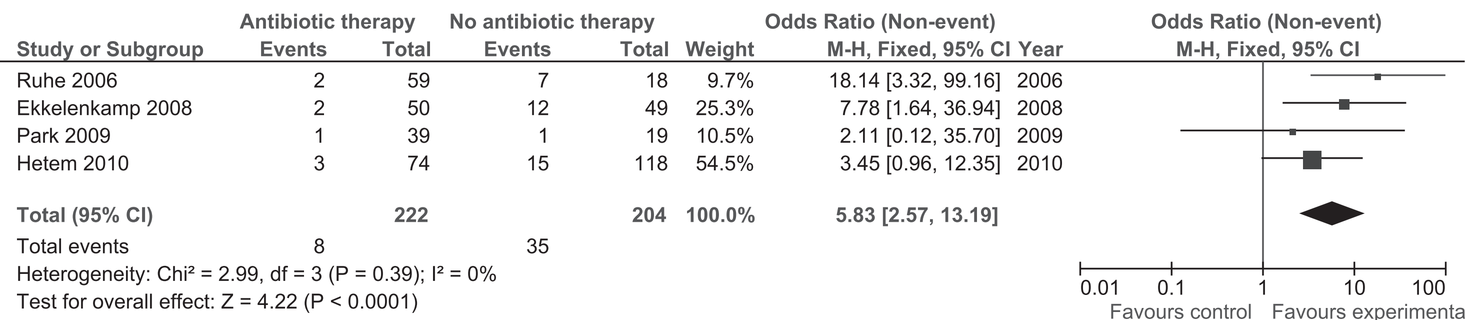
A meta-analysis of the 4 studies on this subject[3,4,7] confirmed the relation between not receiving antibiotic therapy and subsequent SAB with a pooled OR of 5.8; prompt antibiotic therapy led to an absolute risk reduction of 13.6%. These studies were retrospective, and blood cultures were not taken in a protocoled manner to exclude SAB at the time of removal. It is, therefore, possible that a number of patients were in fact already experiencing SAB at the time their IV catheters were removed, but with clinical symptoms too limited (or not recognized) to urge the treating physicians to draw blood cultures and initiate antibiotic therapy. However, in all 4 studies a number of patients did have negative blood cultures between the time the catheter was removed and the time SAB was demonstrated, indicating that negative blood cultures do not exclude later bacteremic complications.

Furthermore, the 4 studies on this subject were limited by inclusion bias: the decision to culture catheter tips was made by clinicians, probably based on clinical signs and symptoms. This may have led to an overestimation of the risk of subsequent SAB. On the other hand, as Table 2 shows, initiation of antibiotic treatment was not random. Clinical symptoms influenced the decision whether a positive catheter tip was treated with antibiotics. Patients with signs and symptoms of infection were more likely to receive therapy, which may have biased toward less observed protective effect of antibiotic therapy.

Table 3. Risk factors for subsequent *Staphylococcus aureus* bacteremia in patients with *S. aureus* colonization of intravascular catheters, combined analysis with the study by Ekkelenkamp e.a.[1].

Variable	Patients without subsequent bacteremia (n=259)	Patients with subsequent SAB after 48h. (n=32)	p-value (univariate analysis)	OR (95%CI)	p-value (multivariate analysis)	OR multivariate analysis (95% CI)
No antibiotic therapy within 24 hours	119 (46%)	5 (16%)	0.001	0.22 (0.08 – 0.58)	0.001	5.4 (2.0 - 15.1)
Documented exit-site infection	85 (33%)	17 (53%)	0.023	2.32 (1.11 – 4.87)	0.003	3.31 (1.5 - 7.4)
Corticosteroid therapy	46 (18%)	12 (37%)	0.007	2.87 (1.30 – 6.32)	0.013	2.9 (1.3 - 6.6)
Immunosuppressive therapy (all)	63 (24%)	15 (47%)	0.007	2.74 (1.30 – 5.81)	0.61	1.4 (0.3 - 5.6)

95% CI: 95% confidence interval, OR: odds ratio.



Study or Subgroup	Antibiotic therapy Events	Antibiotic therapy Total	No antibiotic therapy Events	No antibiotic therapy Total	Weight	Odds Ratio (Non-event) M-H, Fixed, 95% CI	Year
Ruhe 2006	2	59	7	18	9.7%	18.14 [3.32, 99.16]	2006
Ekkelenkamp 2008	2	50	12	49	25.3%	7.78 [1.64, 36.94]	2008
Park 2009	1	39	1	19	10.5%	2.11 [0.12, 35.70]	2009
Hetem 2010	3	74	15	118	54.5%	3.45 [0.96, 12.35]	2010
Total (95% CI)		**222**		**204**	**100.0%**	**5.83 [2.57, 13.19]**	
Total events	8		35				

Heterogeneity: $Chi^2 = 2.99$, $df = 3$ ($P = 0.39$); $I^2 = 0\%$
Test for overall effect: $Z = 4.22$ ($P < 0.0001$)

Figure 1. Meta-analysis of studies on the protective effect of prophylactic antibiotic therapy for IV catheters colonized with *Staphylococcus aureus* to prevent subsequent *Staphylococcus aureus* bacteremia. Comparison: antibiotic therapy for patients with *S. aureus* IV catheter colonization; outcome: SAB. Note: In the studies by Ruhe et al and Park et al, antibiotic therapy was initiated within 48 hours; in the studies by Ekkelenkamp et al and Hetem et al, antibiotic therapy was initiated within 24 hours. M-H = Mantel-Haenszel analysis.

In the current study, we did not take into account whether the antibiotic therapy was specifically targeted to the *S. aureus* cultured from the IV catheter, as long as the agent was active against the cultured isolate. In many cases broad-spectrum antibiotic therapy was started, likely because of systemic symptoms of infectious disease. Most of the patients (53/74, 72%), however, were treated with specific anti-staphylococcal antibiotics, which suggests that their therapy was targeted to the *S. aureus* cultured from the IV catheter.

The IDSA guidelines for diagnosis and treatment of IV catheter-associated sepsis now advocate 5-7 days antibiotic therapy for all patients with *S. aureus* cultured from IV catheter tips, level of evidence B-II (B: moderate evidence to support a recommendation; II: evidence from ≥1 well-designed non-randomized clinical trial, from cohort or case-controlled analytic studies, from multiple time-series, or dramatic results from uncontrolled experiments).[5] This IDSA recommendation is strongly supported by the findings in the current study, which demonstrate its validity for patients in non-university hospitals, and almost double the number of patients the recommendation is based on. Although the IDSA recommendations would be best supported in a randomized controlled trial comparing protocoled antibiotic therapy for IV catheter tip colonization with standard of care treatment, the calculated number needed to treat of 7.4 patients to prevent 1 subsequent case of SAB and the severity of this complication make it practically impossible that such a trial will ever be performed, due to ethical considerations. Additional comparative studies will, therefore, necessarily be retrospective cohort studies.

Based on the results of the current study and our general experience, the protocol at the hospitals where this study was performed is now the following: When *S. aureus* colonization of a catheter tip is demonstrated, intravenous anti-staphylococcal treatment should be started promptly. The local epidemiology of *S. aureus* resistance determines the empiric antibiotic therapy of choice: vancomycin (15 mg/kg q12h) in hospitals where MRSA is frequent or if a patient is colonized with MRSA (alternatives may be daptomycin 6 mg/kg per day, cotrimoxazole 960 mg q12h, or linezolid 600 mg q12h), a β-lactam antibiotic (for example, flucloxacillin/oxacillin/nafcillin 1000 mg q4h, or cefazolin 2000 mg q8h) in hospitals where MRSA is rare, or when a patient is colonized by methicillin-susceptible *S. aureus*. Blood cultures should be drawn before initiating antibiotic therapy. If after 72 hours of intravenous therapy the patient is afebrile and blood cultures remain negative, we believe a switch to an oral antibiotic agent for the remainder of a total of 7 days of treatment is acceptable; antibiotics with high oral resorption should be used (for example, cephalexin 1000 mg q8h, clindamycin 600 mg q8h, cotrimoxazole 960 mg q12h, or linezolid 600 mg q12h). If blood cultures become positive with *S. aureus*, the patient should be treated for 14 days according to the IDSA guidelines.

REFERENCES

1. Fowler VG, Jr, Olsen MK, Corey GR, et al. Clinical identifiers of complicated staphylococcus aureus bacteremia. *Arch Intern Med*. 2003;163(17):2066-2072.
2. Fowler VG, Jr, Justice A, Moore C, et al. Risk factors for hematogenous complications of intravascular catheter-associated staphylococcus aureus bacteremia. *Clin Infect Dis*. 2005;40(5):695-703.
3. Ekkelenkamp MB, van der Bruggen T, van de Vijver DA, Wolfs TF, Bonten MJ. Bacteremic complications of intravascular catheters colonized with staphylococcus aureus. *Clin Infect Dis*. 2008;46(1):114-118.
4. Ruhe JJ, Menon A. Clinical significance of isolated staphylococcus aureus central venous catheter tip cultures. *Clin Microbiol Infect*. 2006;12(9):933-936.
5. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the infectious diseases society of america. *Clin Infect Dis*. 2009;49(1):1-45.
6. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med*. 1977;296(23):1305-1309.
7. Park KH, Kim SH, Song EH, et al. Development of bacteraemia or fungaemia after removal of colonized central venous catheters in patients with negative concomitant blood cultures. *Clin Microbiol Infect*. 2010;16(6):742-746.

eleven

GENERAL DISCUSSION

Staphylococcus aureus is a major cause of severe nosocomial infections associated with considerable morbidity, mortality and health-care costs.[1,2] Transmission of antibiotic resistant *S. aureus* strains in hospital settings, in particular of methicillin-resistant *S. aureus* (MRSA), conveys a risk to hospitalized patients. The occurrence of nosocomial MRSA infections adds to the total burden of disease rather than replacing their methicillin-susceptible *S. aureus* (MSSA) counterparts.[3]

During the recent decades the worldwide epidemiology of MRSA has changed dramatically. New MRSA clones have emerged infecting healthy people without any previous history of healthcare exposure. These so-called community-associated MRSA (CA-MRSA) strains are replacing the health-care associated clones (HA-MRSA) in the United States, with the major dominant clone being USA300.[4] The rapid dissemination of USA300 in hospitals in the USA combined with the high attack rate during outbreaks suggests that this strain has a higher transmission capacity than HA-MRSA strains.

In addition to these CA-MRSA clones a new MRSA clone associated with livestock (sequence type (ST) 398), especially pigs and veal calves, has arisen in multiple countries around the world, including countries with a traditionally low MRSA prevalence.[5]

In the Netherlands we use the so-called "search and destroy" policy for controlling MRSA infection or colonization within hospitals and long-term care facilities. It is composed of the pre-emptive isolation of patients at risk for MRSA colonization, by the strict isolation of known MRSA carriers and the eradication of MRSA carriage in patients and healthcare personnel.[6] Furthermore, contact screenings of patients and personnel are performed after the identification of an unexpected MRSA patient. Mupirocin is a topical antibiotic and the cornerstone for the decolonization of MRSA and MSSA in patients and health-care personnel. Resistance against mupirocin would greatly reduce the effectiveness of these regimens.[7]

Nosocomial transmission of methicillin-resistant *Staphylococcus aureus* (part I)

In **chapter 2** we determined that livestock-associated MRSA genotypes (LA-MRSA), compared with other MRSA genotypes, were 4.4 times less likely to spread in Dutch hospitals. We used data from 62 hospitals in the Netherlands, comprising of 372 months of Dutch MRSA policy, to calculate strain specific $R_{A,}$ the average number of secondary cases that would be caused by an index case during one single hospital admission. The results found were comparable to previous estimates.[8] Absence of significant differences in age, length of hospital stay, or number of days not spent in isolation between index case-patients with LA-MRSA and those with other MRSA reduces the possibility that the differences in transmission capacity resulted from differences in patient characteristics. Patients colonized with LA-MRSA were more likely to be male, probably reflecting sex distributions among pig farmers and veal calf farmers. In this study, we collected more detailed patient information,

such as admission and discharge dates and the number of days that index and secondary case-patients were admitted without barrier precautions, which enabled more precise estimation of parameters in comparison to the previous estimate. Furthermore, all isolates were genotyped, revealing significant heterogeneity in the patients colonized with non LA-MRSA strains. Almost 25% of the non-livestock MRSA isolates carried genes encoding for Panton-Valentine leukocidin (*pvl*) positive, a cytotoxin associated with CA-MRSA clones.

Whole-genome analyses of multiple LA-MRSA strains suggests that these strains might have originated from MSSA that crossed the species barriers from humans to livestock, where it acquired its resistance traits.[9] It has been hypothesized that the transition from humans to animals was associated with the loss of several human immune evasion genes, which may prevent adaptation of LA-MRSA to the human niche.[10] Re-acquisition of these evasion genes could, in theory, lead to an increased transmission capacity between humans.

The R_A values of LA-MRSA found in **chapter 2** study might be an overestimation, as *spa* typing has limited discriminatory power in LA-MRSA considering the high-level of homogeneity within livestock-associated *spa* types (63% *spa* type t011). By using whole genome mapping Bosch et al. revealed that LA-MRSA isolates presumably belonging to the same nosocomial outbreak, were unrelated to each other.[11] All of these isolates had identical *spa* types. Transmission of LA-MRSA from colonized veterinarians to household members appears to be lower compared to transmission from patient colonized with non LA-MRSA, providing additional proof of the reduced human-to-human transfer capacity.[12]

eleven

The Dutch National guideline for MRSA recommends screening all patients with professional exposure to livestock for MRSA colonization, and many hospitals treat such patients in pre-emptive isolation pending the screening results.[6] A previous multicenter study in the Netherlands demonstrated the cost-effectiveness and safety of not pre-emptively isolating patients when using rapid diagnostic testing.[13] That evaluation included all MRSA genotypes: LA-MRSA and non LA-MRSA. The results from **chapter 2** and the results of the previous studies provide evidence that pre-emptive isolation may not be necessary for patients suspected of carrying LA-MRSA, which increases the feasibility of this highly successful infection control policy in areas with a high density of pigs and pig farmers.

In contrast to LA-MRSA and hospital-associated MRSA, there are no established risk factors in the Netherlands for colonization with CA-MRSA, and unknown carriers of these genotypes will not be screened when admitted to a hospital.

In **chapter 2** we demonstrate that PVL-positive strains do not constitute a major risk for health care settings in the Netherlands because the introduction rate and the R_A in the absence of barrier precautions (R_A for PVL-positive strains 0.31, 95% CI 0.14–0.58) are low.

These findings are strengthened by the results found in **chapter 4** in which the transmission capacity of CA-MRSA is determined in Danish hospitals. Using observational data of patients colonized or infected with different genotypes of MRSA in four Danish hospitals and the results of subsequent contact investigations, we concluded that CA-MRSA

was 9.3 times less transmissible than HA-MRSA in Danish hospital setting. We used the same mathematical model based on queuing theory as used in chapter 2 to determine R_A and MRSA strain specific transmission rates.[14] The difference in the R_A values between CA-MRSA and HA-MRSA isolates could result from differences in host factors, bacterial characteristics or a combination of both. Differences in patient age and length of hospital stay were found, suggesting that patients infected with or carrying CA-MRSA differ from those with HA-MRSA; index patients with CA-MRSA were younger and were discharged faster, which suggests that they were in a better health condition. This is in accord with the general conception that CA-MRSA more frequently affects healthy individuals without a history of healthcare exposure. Furthermore, CA-MRSA more frequently caused skin and soft tissue infections (SSTIs), which could have accelerated the detection and implementation of isolation precautions. Lastly, it is possible that these patients are less likely to spread MRSA and that those who share their hospital room are less susceptible to acquisition, due to differences in the severity of illness of the patient and their roommates.

The comparability of the R_A ratios of community genotypes found in Denmark and livestock genotypes found in the Netherlands is hard to determine. Differences in healthcare systems, nursing and ward protocols between countries can be important factors that influence R_A. The R_A of LA-MRSA, CA-MRSA and HA-MRSA found in **chapter 2** and **chapter 4** (and its 95% CI) were all <1, implying that a single admission of a patient colonized with MRSA is unlikely to initiate an epidemic irrespective of the genotype, which confirms previous estimates of the R_A of HA-MRSA. (Cooper, PNAS, Kajita Okano, Nat Rev) Nevertheless, if more CA-MRSA colonized patients get admitted to the hospital, thereby increasing the admission rate, outbreaks could emerge despite R_A values <1.

In **chapter 3** we demonstrate that transmission of MRSA after short-term exposure of MRSA-carriers to the health care setting is rare. Data from 39 hospitals during 6 months revealed that in only one of 111 post-contact screenings performed, two secondary cases were identified.

As only one transmission event was identified in this study any comparison between different genotypes is impossible. In the current Dutch National MRSA guidelines, performing post contact screening after MRSA-exposure in the outpatient clinic is not recommended. Based on the results from this study, these recommendations are justified.

In **chapter 5** we performed a descriptive post-hoc analysis of a multinational prospective study in 13 European intensive care units (ICU's) across 8 countries and revealed that the molecular epidemiology of MRSA was homogeneous within, but heterogeneous between countries. Furthermore, CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were rarely detected among patients admitted to these European ICU's. The homogeneity of sequence types between ICUs within the same country may result from the geospatial distribution of ICUs, as ICU's from the same country were located closely to one another. A regional distribution of MRSA in Europe has been described previously.[15] Our findings

confirm these previous findings, but extrapolation of our results of 13 individual ICUs in 8 different countries is not possible.

The differences in the epidemiology of CA-MRSA in Europe and the United States are not well understood, and more detailed analyses are warranted. The successful emergence of CA-MRSA, USA300 in particular, in hospitals in the US seems contradictory to the low transmissibility of CA-MRSA found in **chapter 4** and the low prevalence found in **chapter 5**. Differences between healthcare settings including general infection control practices and other variables influencing transmission from non-isolated patients (such as contact rates, staffing levels, beds per room, room size and patient turn-around time) will influence the transmissibility of CA-MRSA. Finally, the characteristics of patients colonized or infected with CA-MRSA could be different between US and European hospitals, and this would also influence R_A values.

Only three LA-MRSA were detected in the current study from **chapter 5**, probably because all ICUs participating were situated in urbanised areas. LA-MRSA is predominantly found in rural areas with a high density of pigs and pig farmers. (ref spatial location LA-MRSA.[16]

Dynamics of mupirocin resistance in *Staphylococcus aureus* and coagulase negative staphylococci

Mupirocin is a topical antibiotic used for decolonization of methicillin-susceptible *S. aureus* (MSSA) and MRSA, both in patients and in healthcare personnel, and for treatment of local skin and soft tissue infections caused by *S. aureus* and streptococcal species. In the review described in **chapter 6**, we have summarized the evidence that mupirocin resistance, both high and low level resistance, reduces the effectiveness of decolonizing strategies for *S. aureus* or MRSA. We combined the results from five studies, and found that successful MRSA decolonization was achieved in only 24% of 84 patients with a high-level mupirocin resistant *S. aureus*, which was comparable to a decolonization rate of 29% (of 103 patients) from three studies with patients colonized with low-level mupirocin resistant *S. aureus*. Increased use of mupirocin, especially unrestricted use and treatment of wounds and pressure sores, has been associated with emerging resistance. Emergence of mupirocin resistance during short-term use of mupirocin, such as during outbreak management of MRSA and MSSA is rare.[17] Increasing resistance against mupirocin following the implementation of peri-operative *S. aureus* decolonization has not yet been reported. In 12 studies reporting both the efficacy of mupirocin for *S. aureus* decolonization and the number of isolates acquiring mupirocin resistance, resistance was documented in six of 741 patients (0.8%).[18]

In **chapter 7** we report an increase of highly mupirocin-resistant coagulase negative staphylococci (CoNS) in bloodstream isolates during the 2006-2011 period, all linked to the presence of the plasmid based *mupA* gene. The increase of high-level mupirocin

resistance CoNS was only weakly associated with increased in-hospital use of mupirocin. We found a correlation between high-level mupirocin resistance in CoNS and co-resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, doxycycline, and clindamycin, which confirmed previous observations.[19] As CoNS are frequent causes of prosthetic infections co-resistance can potentially complicate the effective treatment.

Universal decolonization, as in treating all patients irrespective of *S. aureus* carrier ship status, may facilitate emergence of mupirocin resistance in *S. aureus* and CoNS. In **chapter 8** we investigated the effects of peri-operative universal decolonization with topical mupirocin and chlorhexidine body washings on resistance development in CoNS and *S. aureus*. In this prospective cohort study the prevalence of high-level resistance against mupirocin among CoNS was 21% at admission and this increased to 43% after topical treatment with mupirocin and chlorhexidine body washing. Mupirocin-resistant *S. aureus* was only detected in one of 1578 patients at admission and no acquisition of mupirocin resistance within *S. aureus* was observed. In contrast, 37% of the patients with no mupirocin resistance CoNS at admission, acquired mupirocin resistance CoNS following decolonization.

The emergence of high-level mupirocin resistance in CoNS found in **chapter 7** and **chapter 8** indicates an expanding reservoir of plasmids containing the *mupA* gene encoding for high-level mupirocin resistance. These plasmids can, *in vitro* and *in vivo*, be transferred from CoNS to other CoNS strains, to MRSA, and to restriction-deficient *S. aureus* strains.[20,21] The restriction system prevents the interchange of DNA with other bacterial species. In restriction-proficient strains, like most clinical methicillin-susceptible *S. aureus* isolates, horizontal plasmid transfer originating from CoNS strains seems less likely.[20] MRSA, and particularly the epidemic strain USA300, seems to be more susceptible to integration of plasmids carrying *mupA*.[22] Therefore, the low rates of MRSA colonization and infections in the Dutch setting may reduce the generalizability of these findings for MRSA endemic settings. The pre-existing high prevalence and rapid increase of plasmid-based high-level mupirocin resistance in CoNS during treatment warrants careful monitoring of mupirocin resistance development in *S. aureus*. No horizontal gene transfer of *mupA* to *S. aureus* was demonstrated in 936 patients during the 5 days of follow-up. Future studies determining the persistence of carriage with high-level mupirocin-resistant CoNS and quantifying the horizontal transfer rate in patients with longer follow-up are needed for more accurate assessment of the ecological safety of universal decolonization with mupirocin in surgical and critically ill patients.

In **Chapter 9** we compare two different strategies for decolonizing *S. aureus* carriers before surgery: a targeted screening and decolonization strategy versus a universal decolonization strategy. Both strategies for peri-operative decolonization of *S. aureus* carriage were associated with significant health-care gains and cost savings due to prevention of *S. aureus* surgical site infections. Universal decolonization without screening for *S. aureus* carriage was more cost-effective than targeted decolonization based on pre-

operative screening, mainly because of the absence of costs for screening.[23] Yet, these benefits should be balanced against the risk of selecting mupirocin resistance in patients not carrying *S. aureus* on admission. We developed a deterministic mathematical model to describe the dynamics of mupirocin resistance and identify drivers of mupirocin resistance in *S. aureus* in hospital wards. Based on modeling and the literature, we found that the use of mupirocin in patients not carrying *S. aureus* does not extensively increase the risk of emergence of high-level mupirocin resistance in *S. aureus*. However, quantification of the duration of carriage with high-level mupirocin-resistant CoNS and of horizontal gene transfer rates in patients with longer follow-up are needed for more accurate assessment of the ecological safety of universal decolonization with mupirocin in surgical patients. When implementing universal decolonization protocols monitoring of mupirocin susceptibility in *S. aureus* is strongly recommended.

Management of intravascular catheters colonized with *Staphylococcus aureus*

In **chapter 10** it was described that *S. aureus* colonization of intra-venous catheters was complicated by subsequent *S. aureus* bacteremia (>24 h after catheter removal) in 18 of 192 patients without manifest *S. aureus* bacteremia at the time of catheter removal. This complication could be prevented by prompt initiation of antibiotic therapy. The combined results of the 4 studies on this subject confirmed the relation between receiving adequate antibiotic therapy and the risk of developing a subsequent *S. aureus* bacteremia with a relative risk of 0.25 (95% CI, 0.10-0.63). Starting adequate antibiotic therapy within 24 hours led to an absolute risk reduction of 13.6%, which corresponds to a number needed to treat of 7.4 patients to prevent 1 case of subsequent *S. aureus* bacteremia. Based on these results the protocol at the hospitals where this study was performed is now the following: when *S. aureus* colonization of a catheter tip is demonstrated, blood cultures should be drawn, followed by the start intravenous anti-staphylococcal treatment. If the patient is afebrile after 72 hours of intravenous therapy and blood cultures remain negative, a switch to an oral antibiotic agent with good bio-absorption for the remainder of a total of 7 days of treatment is recommended. If blood cultures grow *S. aureus*, a two week treatment according to the IDSA guidelines is recommended.[24]

Future prospects

In this thesis we explored the nosocomial transmissibility of different MRSA clones, the dynamics of mupirocin resistance in staphylococci and the management of colonized IV catheters with *S. aureus*.

The impact of LA-MRSA appears to be low in Dutch hospitals at this moment. The nosocomial transmissibility of LA-MRSA is and remained lower compared to their HA-MRSA counterparts and infections caused by LA-MRSA are rarely seen. In theory, LA-MRSA could potentially re-acquire human immune evasion genes, thereby increasing its transmission

capacity. However, up to this date we have no indication that LA-MRSA transmission capacity or virulence are increasing. For reasons not entirely understood, the increase of CA-MRSA in the USA and USA300 in particular, has not been seen in countries in Europe, including the Netherlands. Introductions of CA-MRSA or *pvl* positive strains occur in Dutch hospitals but are uncommon and at this point in time pose little threat to the low MRSA epidemiology. The high transmission rates of USA300 seen in the community and within households, may eventually increase the number of introductions of CA-MRSA into Dutch hospitals. One could also speculate that an increase in MRSA colonization in healthcare workers could subsequently lead to an increase in transmission in hospitals. Of note, frequent introductions of CA-MRSA in the hospital, even with the low transmissibility found, can still lead to patient-to-patient transmission.

There is a paucity of data on the prevalence of mupirocin resistance and trends in emerging resistance in *S. aureus* from European countries including the Netherlands. Information on the prevalence of resistance at baseline would be important in countries implementing universal decolonization strategies containing mupirocin. Peri-operative universal decolonization is a highly effective prevention strategy for post-operative *S. aureus* surgical site infections, which is associated with extensive cost savings. The benefits of universal decolonization should be weighed against the development of resistance in patients not carrying *S. aureus* at admission. Future studies should determine the persistence of carriage with high-level mupirocin-resistant CoNS. Quantification of the horizontal transfer rate of the *mupA* gene between CoNS and *S. aureus* in patients with longer follow-up is needed for more accurate assessment of the ecological safety of universal decolonization with mupirocin in surgical and critically ill patients.

REFERENCES

1. Wolkewitz M, Frank U, Philips G, Schumacher M, Davey P, BURDEN Study Group. Mortality associated with in-hospital bacteraemia caused by staphylococcus aureus: A multistate analysis with follow-up beyond hospital discharge. *J Antimicrob Chemother*. 2011;66(2):381-386.
2. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant staphylococcus aureus and escherichia coli bacteremia: Estimating the burden of antibiotic resistance in europe. *PLoS Med*. 2011;8(10):e1001104.
3. Ammerlaan HS, Harbarth S, Buiting AG, et al. Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin Infect Dis*. 2013;56(6):798-805.
4. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis*. 2008;46(6):787-794.
5. Fluit AC. Livestock-associated staphylococcus aureus. *Clin Microbiol Infect*. 2012;18(8):735-744.
6. Infection Prevention Working Party. MRSA hospital guideline. http://www.wip.nl/UK/free_content/Richtlijnen/110530%20MRSA%20hospital%20def.pdf. Accessed 05/04, 2015.
7. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in staphylococcus aureus. *J Hosp Infect*. 2013;85(4):249-256.

8. Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant staphylococcus aureus. *J R Soc Interface*. 2011;8(57):578-584.
9. Price LB, Stegger M, Hasman H, et al. Staphylococcus aureus CC398: Host adaptation and emergence of methicillin resistance in livestock. *MBio*. 2012;3(1):10.1128/mBio.00305-11. Print 2012.
10. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005;3(12):948-958.
11. Bosch T, Verkade E, van Luit M, et al. High resolution typing by whole genome mapping enables discrimination of LA-MRSA (CC398) strains and identification of transmission events. *PLoS One*. 2013;8(6):e66493.
12. Verkade E, Kluytmans-van den Bergh M, van Benthem B, et al. Transmission of methicillin-resistant staphylococcus aureus CC398 from livestock veterinarians to their household members. *PLoS One*. 2014;9(7):e100823.
13. Wassenberg MW, Kluytmans JA, Box AT, et al. Rapid screening of methicillin-resistant staphylococcus aureus using PCR and chromogenic agar: A prospective study to evaluate costs and effects. *Clin Microbiol Infect*. 2010;16(12):1754-1761.
14. Trapman P, Bootsma MC. A useful relationship between epidemiology and queueing theory: The distribution of the number of infectives at the moment of the first detection. *Math Biosci*. 2009;219(1):15-22.
15. Grundmann H, Aanensen DM, van den Wijngaard CC, et al. Geographic distribution of staphylococcus aureus causing invasive infections in europe: A molecular-epidemiological analysis. *PLoS Med*. 2010;7(1):e1000215.
16. Feingold BJ, Silbergeld EK, Curriero FC, van Cleef BA, Heck ME, Kluytmans JA. Livestock density as risk factor for livestock-associated methicillin-resistant staphylococcus aureus, the netherlands. *Emerg Infect Dis*. 2012;18(11):1841-1849.
17. Ellis MW, Griffith ME, Dooley DP, et al. Targeted intranasal mupirocin to prevent colonization and infection by community-associated methicillin-resistant staphylococcus aureus strains in soldiers: A cluster randomized controlled trial. *Antimicrob Agents Chemother*. 2007;51(10):3591-3598.

18. Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ. Eradication of methicillin-resistant staphylococcus aureus carriage: A systematic review. *Clin Infect Dis*. 2009;48(7):922-930.
19. Han LL, McDougal LK, Gorwitz RJ, et al. High frequencies of clindamycin and tetracycline resistance in methicillin-resistant staphylococcus aureus pulsed-field type USA300 isolates collected at a boston ambulatory health center. *J Clin Microbiol*. 2007;45(4):1350-1352.
20. Udo EE, Jacob LE. Conjugative transfer of high-level mupirocin resistance and the mobilization of non-conjugative plasmids in staphylococcus aureus. *Microb Drug Resist*. 1998;4(3):185-193.
21. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from staphylococcus epidermidis to methicillin-resistant staphylococcus aureus associated with failure of mupirocin prophylaxis. *J Antimicrob Chemother*. 2005;56(6):1166-1168.
22. Diep BA, Stone GG, Basuino L, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette mec linkage: Convergence of virulence and resistance in the USA300 clone of methicillin-resistant staphylococcus aureus. *J Infect Dis*. 2008;197(11):1523-1530.
23. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and eradication of staphylococcus aureus carriage. *PLoS One*. 2011;6(5):e14815.
24. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant staphylococcus aureus infections in adults and children: Executive summary. *Clin Infect Dis*. 2011;52(3):285-292.

SAMENVATTING IN HET NEDERLANDS

Staphylococcus aureus is een bacterie die bij 20-30% van de bevolking voorkomt op de huid, op de slijmvliezen en vooral in de neus. Mensen die de *Staphylococcus aureus* bacterie bij zich dragen noemen we "dragers". *Staphylococcus aureus* kan een grote verscheidenheid aan infecties veroorzaken: van oppervlakkige huidinfecties, zoals een relatief onschuldige impetigo (krentenbaard) bij kinderen, tot ernstige infecties van de hartkleppen. In het ziekenhuis is *Staphylococcus aureus* de belangrijkste veroorzaker van infecties bij opgenomen patiënten, met name van wondinfecties na een operatie. Eerder onderzoek heeft aangetoond dat patiënten die voorafgaand aan een ziekenhuisopname drager zijn van *Staphylococcus aureus* meer kans hebben op het ontwikkelen van een infectie veroorzaakt door deze bacterie gedurende de ziekenhuisopname.

Een van de onderscheidende eigenschappen van *Staphylococcus aureus* is dat hij snel resistent wordt tegen antibiotica. Midden jaren 40, toen penicilline nog maar sinds enkele jaren op grote schaal werd gemaakt, werd de eerste penicilline resistente *Staphylococcus aureus* gedetecteerd. In de jaren 50 was 40% van de *Staphylococcus aureus* stammen resistent tegen penicilline, hetgeen toenam tot meer dan 80% in de jaren 60. Voor alle antibiotica die na deze periode zijn geïntroduceerd, circuleren nu resistente stammen.

Beta-lactam antibiotica (o.a. penicilline, methicilline, flucloxacilline) zijn de eerste keus voor de behandeling van *Staphylococcus aureus* infecties. Resistentie tegen deze groep van beta-lactam antibiotica, zogenoemde methicilline-resistente *Staphylococcus aureus* of MRSA, is problematisch omdat de eerste keus antibiotica geen effect meer hebben. Alternatieve antibiotica om *Staphylococcus aureus* infecties mee te behandelen zijn vaak minder werkzaam, alleen via een infuus toe te dienen, duurder of hebben meer bijwerkingen.

MRSA komt in Nederlandse ziekenhuizen weinig voor. Patiënten met een hoog risico op MRSA dragerschap (bijvoorbeeld bij een overname uit een buitenlands ziekenhuis) worden in isolatie (met schort, handschoenen en mondkapjes) op een eenpersoonskamer verpleegd tot bekend is of een patiënt wel of geen MRSA drager is. Als een patiënt tijdens een opname onverwacht drager blijkt te zijn van een MRSA-bacterie dan wordt deze patiënt in isolatie gelegd en vervolgens worden patiënten en personeel die in aanraking zijn gekomen met een MRSA-drager gescreend op dragerschap.

Verspreiding van MRSA

Boeren van varkens en vleeskalveren zijn vaker drager van MRSA. Twintig procent van deze boeren zijn drager van een specifieke vee-gerelateerde MRSA stam die geassocieerd is met het houden van deze dieren. Vee-gerelateerde MRSA stammen lijken zich te onderscheiden van andere MRSA stammen doordat ze minder infecties veroorzaken en doordat ze zich minder goed lijken te verspreiden in Nederlandse ziekenhuizen. In **hoofdstuk 2** hebben we de verspreidingscapaciteit van vee-gerelateerde MRSA vergeleken met andere MRSA

stammen in 62 Nederlandse ziekenhuizen. Hieruit bleek dat vee-gerelateerde MRSA stammen zich 4,4x minder goed verspreiden t.o.v. andere MRSA stammen in Nederlandse ziekenhuizen, hetgeen overeenkomt met eerdere studies die hiernaar zijn verricht. Patiënten die drager waren van een vee-gerelateerde MRSA stam waren qua leeftijd en opnameduur in het ziekenhuis vergelijkbaar met patiënten met andere MRSA stammen. Wel was 80% van de dragers van vee-gerelateerde MRSA van het mannelijk geslacht, wat waarschijnlijk een weerspiegeling is van de sekseverdeling onder varkens- en vleeskalverenboeren. Het is mogelijk dat vee-gerelateerde MRSA stammen enkele specifieke genen missen waardoor ze zich niet goed tussen mensen kunnen verspreiden.

In hoofdstuk 3 laten we zien dat wanneer een MRSA drager maar kortdurend ongeïsoleerd contact heeft gehad met personeel en patiënten (bijvoorbeeld bij een poliklinisch bezoek of dagbehandeling) het risico op transmissie uiterst klein is. Gedurende de studieperiode van 6 maanden waren er 111 MRSA dragers die kortdurend, zonder aanvullende hygiëne of isolatiemaatregelen waren gezien op de polikliniek of afdeling, waarbij slechts in één geval transmissie naar een andere patiënt of medewerker werd vastgesteld.

MRSA was tot voor kort een bacterie die alleen in ziekenhuizen voorkwam. Sinds begin jaren 2000 is er een toename van MRSA stammen die voorkomen in gezonde, relatief jonge personen die niet eerder in aanraking zijn geweest met zorginstellingen, zoals ziekenhuizen. In toenemende mate worden deze "community associated" MRSA (CA-MRSA) stammen bij patiënten geïsoleerd. CA-MRSA stammen veroorzaken voornamelijk huid- en weke deleninfecties en zijn veel voorkomend in de Verenigde Staten. In **hoofdstuk 4** laten we zien dat deze CA-MRSA stammen zich bijna 10x minder goed verspreiden in 4 Deense ziekenhuizen dan andere MRSA stammen. Dragers van CA-MRSA waren jonger, en minder lang opgenomen in het ziekenhuis ten opzichte van patiënten die waren gekoloniseerd met ziekenhuis geassocieerde MRSA stammen. Dit is in overeenstemming met eerdere studies die aantonen dat CA-MRSA gezonde mensen infecteert die niet eerder in een ziekenhuis zijn opgenomen. Mogelijk verspreiden CA-MRSA stammen zich minder goed omdat de dragers gezonder zijn en minder invasieve procedures (zoals intraveneuze lijnen en beademing) hoeven te ondergaan.

Mupirocine resistentie

Mupirocine is een antibioticum dat lokaal wordt gebruikt om de *Staphylococcus aureus* bacterie te doden. Mupirocine wordt gebruikt in de neus om *S. aureus* en MRSA dragers te behandelen. Het is gebleken dat als *Staphylococcus aureus* dragers worden behandeld met mupirocine zalf in de neus en chloorhexidine wasbeurten voorafgaand aan een operatie, er minder infecties met *S. aureus* voorkomen in de periode na de operatie. Daarnaast wordt mupirocine gebruikt om MRSA dragers te dekoloniseren. Resistentie tegen mupirocine in *S. aureus* zorgt dat het antibioticum niet meer effectief is en MRSA dragers moeilijker kunnen worden behandeld. In **hoofdstuk 6** wordt de wereldwijde epidemiologie van mupirocine resistentie beschreven, het effect van mupirocine resistentie op een succesvolle *S. aureus*

dragerschapsbehandeling, en wordt de relatie beschreven tussen het toenemende gebruik van mupirocine en toenemende resistentie. In **hoofdstuk 7** is een toename in het gebruik van mupirocine geassocieerd met een toename in resistentie in coagulase negatieve staphylococcen (CNS). CNS zijn huidbacteriën en onderscheiden zich van de *Staphylococcus aureus* bacterie doordat ze geen coagulase kunnen produceren en minder virulent zijn. Het *mupA* gen dat ten grondslag ligt aan resistentie tegen mupirocine is identiek in *S. aureus* en CNS en overdracht van dit gen tussen deze bacteriën is eerder beschreven. Hierdoor zou een toename van mupirocine resistentie in CNS een reservoir van mupirocine resistentie voor *S. aureus* kunnen vormen. In **hoofdstuk 7** wordt er een verband gevonden tussen een toename van mupirocine gebruik in het UMC Utrecht en een toename van resistentie in CNS verkregen uit bloedkweken. Mupirocine resistente CNS waren vaker resistent tegen andere groepen antibiotica zoals de fluorchinolonen, macroliden en lincosamiden. De behandeling van infecties met vreemd lichaamsmateriaal (b.v. kunstheupen), waarin deze antibiotica vaak worden gebruikt, kan hierdoor worden gecompliceerd.

Momenteel worden alle patiënten die worden opgenomen op de cardio-thoracale chirurgie, orthopedie of neurochirurgie van het UMCU behandeld met mupirocine in de neus en chloorhexidine wasbeurten, ongeacht of een patiënt drager is van *S. aureus*. Aangezien maar 20%-30% van alle personen een *S. aureus* bacterie bij zich draagt, wordt 70%-80% van de patiënten onnodig behandeld. In **hoofdstuk 8** hebben we gekeken naar de effecten op het ontstaan van resistentie tegen mupirocine in staphylococcen bij de individuele patiënt, zowel *S. aureus* en CNS. Resistentie tegen mupirocine, veroorzaakt door het *mupA* gen, bleek veel voor te komen bij CNS maar was zeldzaam in *S. aureus*. Bij opname droeg 20% van alle patiënten een mupirocine resistente CNS bij zich. Van de patiënten die bij opname nog geen drager waren van een mupirocine resistente staphylococ, verkreeg 37% een mupirocine resistentie in staphylococcen gedurende 5 dagen. Ondanks dat transmissie van het *mupA* gen tussen *S. aureus* en CNS is beschreven lijkt het in onze studie niet voor te komen. Mogelijk is de follow-up te kort geweest.

In **hoofdstuk 9** hebben we twee strategieën om *S. aureus* dragers voorafgaand aan een operatie te dekoloniseren, met elkaar vergeleken. We vergeleken de effectiviteit en kosteneffectiviteit en het risico op het ontstaan van resistentie tegen mupirocine voor twee strategieën: 1. Gerichte screening van patiënten en dekolonisatie van *S. aureus* dragers: hierin wordt bij iedere patiënt voorafgaand aan de operatie gekeken of deze drager is van *S. aureus* en alleen *S. aureus* dragers worden vervolgens behandeld met mupirocin neuszalf en chloorhexidine wasbeurten, versus 2. Universele dekolonisatie: waarbij iedereen voorafgaand aan de operatie wordt behandeld met mupirocine en chloorhexidine wasbeurten, ongeacht of een patiënt drager is van *S. aureus*. Met behulp van een wiskundig model proberen we daarnaast factoren te identificeren die verspreiding van mupirocin resistentie in *S. aureus* op een ziekenhuisafdeling doen toenemen. Een universele dekolonisatiestrategie waarbij iedereen wordt behandeld ongeacht dragerschap is minstens

even effectief in het voorkomen van postoperatieve *S. aureus* infecties en bespaart kosten t.o.v. een gerichte screening en dekolonisatie strategie.

In **hoofdstuk 10** werd gekeken naar een veelvoorkomende complicatie in ziekenhuizen: de lijninfectie. Infusen en catheters die in een grote ader of slagader worden gelegd (zgn. centrale lijnen) worden gebruikt voor het geven van medicijnen (b.v. chemotherapie of antibiotica) en vocht en kunnen gekoloniseerd raken met *S. aureus*. De bacterie kan via de infuuslijn of centrale lijn makkelijk in de bloedbaan van de patiënt terecht komen. De uiteinden van deze infusen of centrale lijnen worden gekweekt bij verdenking op een lijninfectie. Patiënten waar een *S. aureus* van de lijn werd gekweekt hadden een grote kans op het ontwikkelen van een bloedvergiftiging met *S. aureus* gedurende de ziekenhuisopname. Wanneer patiënten met een gekoloniseerde lijn met *S. aureus* werden behandeld met adequate antibiotica dan traden er minder bacteriële complicaties op zoals bloedvergiftigingen en bot- en gewrichtsinfecties met *S. aureus*. Het advies is nu om bij een patiënt met een gekoloniseerde lijn met *S. aureus* bloedkweken af te nemen en in afwachting van het resultaat hiervan te starten met antibiotica voor 5-7 dagen.

CONCLUSIES

In dit proefschrift wordt de verspreiding van diverse MRSA stammen, het vóórkomen en ontstaan van mupirocine resistentie in staphylococcen en het voorkómen van *S. aureus* infecties bij gekoloniseerde centrale catheters besproken.

Op dit moment verspreiden veegerelateerde MRSA stammen zich minder makkelijk in Nederlandse ziekenhuizen en veroorzaken ze minder infecties. Dit kan in de toekomst veranderen wanneer veegerelateerde stammen zich aanpassen aan de menselijke gastheer maar tot op heden zijn daar geen aanwijzingen voor.

De toename van MRSA buiten ziekenhuizen zoals in de Verenigde Staten wordt vooralsnog, om onduidelijke redenen, niet gezien in Europa en Nederland. Het aantal patiënten dat wordt opgenomen en gekoloniseerd is met MRSA zonder ooit eerder in aanraking te zijn geweest met een gezondheidszorginstelling is klein. Mocht dit in de toekomst toenemen dan kan dat tot problemen leiden wanneer medisch personeel zou worden gekoloniseerd.

Er is een gebrek aan informatie over het vóórkomen van mupirocine resistentie in *S. aureus* in Europa en Nederland. Het is belangrijk om te weten hoeveel mupirocine resistentie voorkomt voordat er wordt gestart met universele dekolonisatiestrategieën, waarbij iedere patiënt wordt behandeld met mupirocine, ongeacht of hij drager is van de *S. aureus* bacterie. Deze strategie om iedereen te behandelen kost het minst en is het effectiefst in het voorkomen van infecties met S. aureus. Deze voordelen moeten worden afgewogen tegen het grootscheepse gebruik en het daaruit voortkomende risico op het ontstaan van resistentie.

DANKWOORD

Het is vijf jaar geleden dat ik startte met mijn opleiding tot arts-microbioloog en tegelijkertijd begon aan mijn promotieonderzoek op de afdeling medische microbiologie in het UMC Utrecht. Dit proefschrift is tot stand gekomen met de hulp van velen, waarvan ik er hier een aantal apart wil bedanken.

Allereerst mijn promotor, Prof. dr. Marc Bonten. Beste Marc, dank dat ik bij jou naast mijn opleiding ook mijn promotieonderzoek heb kunnen doen. Ik heb respect voor de manier waarop jij het leiden van een afdeling combineert met het doen van hoogstaand wetenschappelijk onderzoek en een jong gezinsleven. Dat je ondanks dit drukke leven nog steeds snel reageert op mails, manuscripten redigeert, en dat ik altijd even kon binnenlopen om te overleggen bewonder ik des te meer.

Dr. Martin Bootsma, beste Martin, veel van de artikelen in dit proefschrift zouden niet hebben bestaan zonder jouw hulp. Alle modellen en wiskundige formules komen uit jouw koker. Ik heb onze samenwerking als bijzonder prettig ervaren en hoop in de toekomst ook nog vaak met je te brainstormen over artikelen, analyses en modellen onder het genot van goede koffie.

Prof. dr. Henrik Westh, kære Henrik, thank you for the fruitful collaboration.

Dr. Miquel Ekkelenkamp, beste Miquel, mijn eerste onderzoekservaring deed ik op tijdens mijn onderzoeksstage onder jouw begeleiding. Des te toepasselijker is het dat ik het eerste hoofdstuk van dit proefschrift samen met jou heb kunnen schrijven. Jij was mijn inleiding in het doen van onderzoek én in de microbiologie.

Dr. Lennie Derde, beste Lennie, dank voor de fijne samenwerking. Ik hoop dat we elkaar in de toekomst nog vaak zullen tegenkomen in de kliniek en daarbuiten.

Alle stafleden van het UMC Utrecht en het Diakonessenhuis Utrecht, dank voor de prettige samenwerking.

Alle (ex)-AIOS van de microbiologie, aangezien ik niet bij een specifieke onderzoekslijn was aangesloten en ook niet altijd bij onderzoeksbesprekingen aanwezig kon zijn, waren jullie mijn directe collegae tijdens mijn promotieonderzoek. Ik denk met veel plezier terug aan de congresbezoeken met jullie in Villars, Grindelwald en de verschillende ECCMIDs en hoop jullie ook in de toekomst nog te blijven spreken.

Paranymphen, Firdaus Mohamed Hoesein en Lieke Reubsaet, dank voor jullie hulp tijdens mijn promotie en vooral voor jullie gezelligheid.

Mijn broer Rico, lieve Rico, dank voor je interesse in mijn onderzoek en specialisatie. Ik hoop dat we elkaar ondanks onze totaal verschillende werktijden binnenkort weer wat vaker kunnen zien.

Mijn lieve ouders, zonder jullie was ik niet op de plek waar ik nu ben. Jullie staan altijd voor mij, Isis en onze dochters klaar, en dat te weten is een groot iets.

Mijn laatste woorden zijn voor Isis. Lieve Isis, de hele weg van mijn studie, promotie en specialisatie hebben we samen gelopen. In deze tijd hebben we ook nog eens twee schitterende dochters gekregen. Ik kijk uit naar wat de toekomst ons gaat brengen.

David J. Hetem

CURRICULUM VITAE

David Jelger Hetem was born on the 16th of March 1984 in The Hague, the Netherlands. After completing his secondary school in 2003 at the Stichtse Vrije School in Zeist, he started studying medicine at the University of Utrecht. During his study he took elective courses in Infectious Diseases and did a six month scientific internship at the medical microbiology ward in the UMC Utrecht under supervision of dr. Miquel Ekkelenkamp. In 2010 he obtained his medical degree and subsequently started his specialization in medical microbiology in the UMC Utrecht (supervision of dr. Annemarie Weersink and dr. Anne Wensing) and Diakonessenhuis Utrecht (supervision of dr. Steven Thijsen). At the same time he started his scientific career under supervision of prof. dr. Marc Bonten, resulting in this thesis. During his residency he did a research project in collaboration with prof. dr. Henrik Westh at Hvidovre hospital in Denmark. He is currently in his final year of his residency in the UMC Utrecht. He lives with Isis Butôt and their two children Raewyn and Vesper.

David Jelger Hetem werd geboren op 16 maart 1984 te Den Haag. Na het behalen van zijn VWO-diploma op de Stichtse Vrije School in Zeist in 2003 begon hij met zijn studie geneeskunde aan de Universiteit Utrecht. Gedurende zijn studie geneeskunde volgde hij het keuzevak Infectieziekten en volgde hij zijn wetenschappelijke stage op de afdeling medische microbiologie in het UMC Utrecht onder supervisie van dr. Miquel Ekkelenkamp. In 2010 behaalde hij zijn artsenbul en in datzelfde jaar begon hij met zijn opleiding tot arts-microbioloog in het UMC Utrecht (opleiders: dr. Annemarie Weersink en dr. Anne Wensing) en het Diakonessenhuis Utrecht (opleider: dr. Steven Thijsen). Naast zijn opleiding tot arts-microbioloog begon hij in 2010 met zijn promotieonderzoek onder supervisie van prof. dr. Marc Bonten, wat heeft geresulteerd in dit proefschrift. Tijdens zijn opleiding heeft hij een periode onderzoek gedaan in het Hvidovre Ziekenhuis te Kopenhagen onder begeleiding van prof. Henrik Westh. Hij woont samen met Isis Butôt en hun twee kinderen: Raewyn en Vesper.

LIST OF PUBLICATIONS

Hetem DJ, Vogely HC, Severs TT, et al. Acquisition of high-level mupirocin resistance in CoNS following nasal decolonization with mupirocin. *J Antimicrob Chemother*. 2015 Apr;70(4):1182-4

Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. *J Hosp Infect*. 2013 Dec;85(4):249-56.

Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis*. 2013 Nov;19(11):1797-1802.

Hetem DJ, Pekelharing M, Thijsen SF. Probable transmission of *Yersinia enterocolitica* from a pet dog with diarrhoea to a 1-year-old infant. *BMJ Case Rep*. 2013 Aug 16;2013. pii: bcr2013200046.

Bathoorn E, **Hetem DJ**, Alphenaar J, Kusters JG, Bonten MJ. Emergence of high-level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. *J Clin Microbiol*. 2012 Sep;50(9):2947-50.

Hetem DJ, Westh H, Boye K, et al. Nosocomial transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Danish Hospitals. *J Antimicrob Chemother*. 2012 Jul;67(7):1775-80.

Hetem DJ, de Ruiter SC, Buiting AG, et al. Preventing *Staphylococcus aureus* bacteremia and sepsis in patients with *Staphylococcus aureus* colonization of intravascular catheters: a retrospective multicenter study and meta-analysis. *Medicine (Baltimore)*. 2011 Jul;90(4):284-8.

Hetem DJ, Woerdeman PA, Bonten MJ, Ekkelenkamp MB. Relationship between bacterial colonization of external cerebrospinal fluid drains and secondary meningitis: a retrospective analysis of an 8-year period. *J Neurosurg*. 2010 Dec;113(6):1309-13.