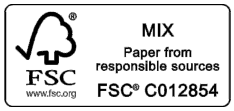
Staphylococcus aureus and healthcare-associated infections

Miquel Bart Ekkelenkamp



***Staphylococcus aureus* and healthcare-associated infections**

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Staphylococcus aureus and healthcare-associated infections

***Staphylococcus aureus* en gezondheidszorg-geassocieerde infecties**

(met een samenvatting in het Nederlands)

***Staphylococcus aureus* e infecciones hospitalarias**

(con un resumen en Español)

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Contents of this thesis

I

Thesis outline

Ever since our earliest ancestors managed to outrun other predators, the main cause of human death has always been infectious diseases: the endemic diseases constantly thinning our numbers; the occasional plague sweeping over the continents and wiping out civilizations. Over time, however, and in particular over the last century, increased hygiene, antibiotics and vaccines and have managed to gradually decrease the burden of infectious diseases. In combination with industrialized agriculture and a lack of armed conflicts this has increased the life-expectancy in the Western world to the point that we have started suffering from historically rare afflictions such as heart-disease and cancer.

Death from community-acquired infectious is becoming a vague memory. Maybe it is this sporadic confrontation with infectious diseases which has made us so prone to panic and hysteria over the slightest chance of acquiring infections, sometimes even imaginary infections. Literally billions of euros are spent each year on all sorts of unproven interventions trying to reduce immeasurable risks of improbable transmissions further to an unachievable zero. Astronomical amounts are spent on stockpiling anti-viral agents and vaccines for fantasy-influenza, on leukocyte-depletion of blood products to prevent transmission of variant Creutzfeld-Jacob disease (which has never occurred), on screening patients for transmission of HIV or hepatitis through suboptimally washed endoscopes (which has never occurred either), on removing non-pathogenic bacteria from our water supply.

The fear for existent healthcare-associated infections bleaks in comparison with that for politically-bloated non-existent ones. Undeservingly so: wound infections, intravascular-catheter associated bloodstream infections, orthopedic implant infections, hospital-acquired

pneumonia's, secondary meningitis, infections in the medically immunocompromized host, prosthetic heart valve endocarditis, CAPD-itis… The chances of getting eaten by another predator are still quite low, but apart from that the risks we expose our patients to quite resemble those of our bearskin-wearing forefathers.

The high prevalence of *Staphylococcus aureus*, in combination with its propensity to infiltrate tissues, colonize foreign body material, form abscesses and produce toxins, makes it by far the most dreaded micro-organism in healthcare-associated infections. There is not a single disease in the list above where it does not rank among the top three of causative pathogens.

This thesis will focus on areas of improvement for healthcare-associated *S. aureus* infections. It will explore topics which have over the years received far less attention than they would merit, and look at that which we always wanted to know, but never dared research.

After a basic introduction into the micro-organism in chapter II, chapter III will discuss a case of a healthcare-associated infection with *S. aureus* with a pig-associated strain. Chapter IV will examine the relation between *S. aureus* bacteremia and *S. aureus*. Chapters V and VII will dig into the risks associated with *S. aureus* colonizing a catheter or drain inserted into a sterile part of the patient (i.e. the bloodstream or the cerebrospinal fluid space), and chapter VI will focus on how to diagnose meningitis in neurosurgical patients. *S. aureus* catheter-associated bloodstream infection can be a rapidly fatal, and its treatment depends on two major interventions: extraction of the infected IV-catheter and initiation of antibiotic therapy. In chapter VIII an attempt will be made to quantify the mortality associated with delay in adequate treatment.

Finally, the constant increase in antibiotic resistance of both hospital-acquired and community-acquired *S. aureus* has necessitated the development of new drugs; chapter IX will deal with the search for compounds active against *S. aureus*, which hopefully one day may lead to the development of novel antibiotics.

II

Staphylococcus aureus

Miquel B. Ekkelenkamp, Suzan H.M. Rooijakkers, Marc J.M. Bonten

Infectious Diseases 3rd edition, edited by Cohen, Powderly and Opal:
Chapter 165, Staphylococci and micrococci

Introduction

Historically, based on morphological similarities, the genera *Staphylococcus*, *Micrococcus*, *Planococcus* and *Stomatococcus* were placed together in the family Micrococcaceae. More recently, however, based on DNA/ rRNA-analysis and GC-content, the genus *Staphylococcus* has been classified together with the genera Bacillus, Brochothrix, Gemella, Listeria and Planococcus, in the family *Bacillaceae* of the broad Bacillus-Lactobacillus-Streptococcus cluster of Gram-positive bacteria with a low GC-content.[1-3]

Staphylococci, in particular *Staphylococcus aureus*, are frequent causes of infection in humans. Next to *S. aureus*, the foremost pathogenic staphylococcal species are *S. lugdunensis*, *S. schleiferi*, *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus*. *S. lugdunensis* and *S. schleiferi* may cause infections similar to those caused by *S. aureus*, including abcesses, endocarditis and wound infections. *S. epidermidis* is primarily responsible for foreign body infections, and both prosthetic and native valve endocarditis. *S. saprophyticus* is a cause of urinary tract infections. Multiple animal species can be colonized or infected by different staphylococcal species. *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.

Nature

History

The name "staphylococcus" (derived 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.[4] In a series of laboratory experiments and clinical observations he subsequently described staphylococcal disease and its role in sepsis and abscess formation. 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*, and a species with white colonies which he called *Staphylococcus albus* and that was later renamed *Staphylococcus epidermidis*.

Staphylococcal infections appeared to play a major role in wound infections. As the cause of post-influenza necrotizing pneumonias, *S. aureus* was considered responsible for a quarter to a third of the deaths during the Spanish flue pandemic of 1917-1918.[5] Also, 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*. Such was the impact of *S. aureus* infections on military campaigns that during the second world war the production process for penicillin (then still universally active against the bacterium) was considered a military secret by the allied forces. Even now, in the antibiotic era, *S. aureus* continues to be a major community-acquired pathogen and the single most relevant cause of nosocomial infections.

Other staphylococci, mainly *S. epidermidis*, are important pathogens in immunocompromized patients and patients with prosthetic materials and indwelling catheters.

Microbiology

Staphylococci are Gram-positive spherical bacteria about 1 micrometer in diameter, which divide in two planes and, therefore, grow in clusters; they are non-motile, non-spore-forming, and have a genome size of between 2000 and 3000 kbp, with a 30-39% GC-content. In 2007, thirty-nine species were recognized within the genus Staphylococcus, seventeen of which were isolated from humans; new species continue to be discovered (www.bacterio.cict.fr). Most staphylococcal species demonstrate catalase activity and are facultative anaerobes. Further characteristics of the genus include susceptibility to furanzolidone, 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 (figure 1), 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 crosslinked 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 and to the cytoplasmic membrane. The polysaccharides are species specific; ribitol teichoic acids are present in *S. aureus* cell walls and glycerol teichoic acids are present in *S. epidermidis*.

Pathogenicity

The broad spectrum of diseases caused by *S. aureus* is associated with its production of many surface-bound and extracellular virulence factors. The bacterium has a large variety of molecules that specifically counteract the host defence mechanisms, and it excretes a number of toxins with membrane damaging, pyrogenic or epidermolytic actions (Table 1A-C).

Adherence to host cells and tissues

S. aureus expresses a number of adhesion molecules that facilitate interactions with host cells and extracellular matrix (ECM) components allowing effective colonization. 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.[6] The collagen-binding adhesin, for instance, recognizes collagen type I and IV and was shown to play an important role in the pathogenesis of septic arthritis induced by *S. aureus*. MSCRAMMS are also involved in the attachment of *S. aureus* and *S. epidermidis* to foreign body materials and indwelling devices: coating of the biomaterials with a mixture of host proteins and platelets subsequently leads to biofilm formation.[7]

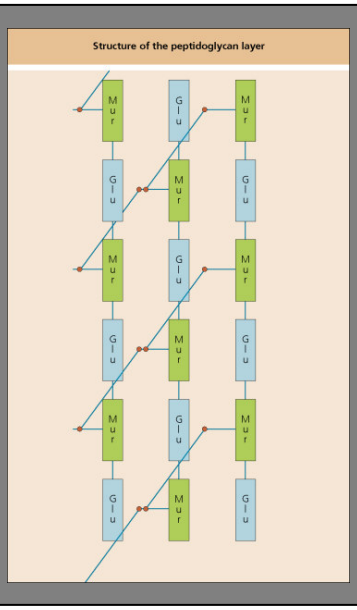


Figure 1: Structure of the peptidoglycan layer.
The peptidoglycan layer consists of three integral parts. The glycan chains are built with 10-12 altenrating *N*-acetylglucosamine (Glu) and *N*-acetylmuramic acid (Mur) subunits jointed with β-1,4 glycosidic bonds. Vertical pentapeptide side chains are linked to the muramic acids subunits, and the side chains are in turn cross-linked with diagonal intrapeptide bridges. For example, the glycan chains in *Staphylococcus aureus* are cross-linked with pentaglycine bridges attached to L-glycine in one pentapeptide chain and D-alanine in an adjacent chain.
Courtesy of Jan Verhoef, Ad C Fluit & Franz-Josef Schmitz**.**

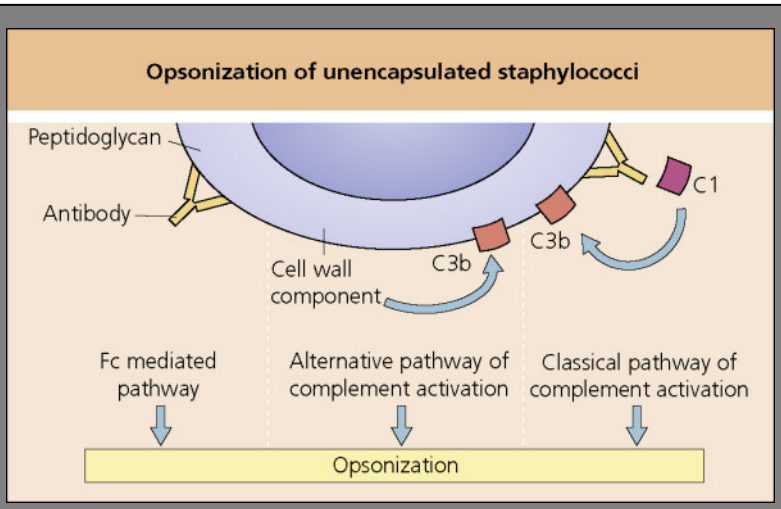


Figure 2: Opsonization through complement activation is primarily a function of C3b and iC3b.
When antibody (ab) molecules bind to antigen, the antigen–antibody complex activates the first complement component, C1. C1 is then converted into an esterase, initiating the classical pathway. Additionally, some cell-wall components can activate the alternative pathway. Courtesy of Jan Verhoef, Ad Fluit & Franz-Josef Schmitz**.**

Table 1A: Immune evasion mechanisms

Virulence factor	*Acronym/gene*	*Activity*
Clumping factor (bound coagulase)	ClfA & ClfB	Binds fibrinogen, coating the bacterial cell and inhibiting phagocytosis
Chemotaxis Inhibitory Protein of *Staphylococcus 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 leucocyte adhesion, diapedesis and extravasation
Extracellular fibrinogen-binding protein/ Extracellular complement binding protein	Efb/Ecb	Binds to the C3d part of C3 molecules, inhibiting C3b containing convertases Efb also binds fibrinogen
Staphylococcal Complement Inhibitor	SCIN/ SCIN-C	Binds and stabilizes C3 convertases, inhibiting C3b deposition and phagocytosis
Staphylokinase	SAK	Activates human plasminogen at the bacterial surface, leading to degradation of IgG and C3b; inhibits bactericidal effect of α-defensins
Polysaccharide capsule		Antiphagocytic function
FLPR1 inhibitory protein	FLIPr	Impairs neutrophil responses to formyl 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: inactivates hydrogen peroxidase and free radicals formed by the myeloperoxidase system of phagocytic cells
Protein A	SpA	Binds Fc part of human IgG and prevents phagocytic uptake by Fc receptors; Stimulates B lymphocytes
Free coagulase	*coa*	Coats the bacterial cell with fibrinogen and inhibits phagocytosis
Staphylococcal Superantigen Like 5	SSL5	Binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling
Staphylococcal Superantigen Like 7	SSL7	Binds IgA and blocks FcalphaRI-mediated responses Binds C5 and blocks C5 cleavage into C5a and C5b
Aureolysin		Metalloproteinase that cleaves LL-37
Staphylococcal immunoglobulin-binding protein	SBI	Binds IgG and C3 Blocks complement activity

Table 1B: invasion mechanisms

Virulence factor	*Gene*	*Activity*
α-hemolysin	*hla*	Multimerizes on eukaryotic membranes to from lytic pores
β-hemolysin	*hlb*	Sphingomyelinase, damages eukaryotic cell membranes containing sphingomyelin by enzymatic alteration of their lipid content; causes lysis of sheep-erythrocytes on blood agar
γ-hemolysin	*hlgA &hlgB*	Two proteins which assemble as membrane-perforating complexes; toxic to PMNLs, monocytes and macrophages, lytic for RBCs
Panton-Valentine Leucocidine	*lukS (lukS-PV, lukF-PV)*	Homologue of γ-hemolysin encoded by mobile phage, lytic to leucocytes; associated with furunculosis and necrotizing pneumonia
Leucocidin E-D	*LukED*	Lytic to leucocytes
δ- hemolysin	*hld*	Variety of attributed actions, such as formation of lytic pores on eukaryotic membranes and mediator of staphylococcal membranous enterocolitis
Exfoliative toxins	*eta & etb*	Toxins with protease activity; epidermolytic effect on stratum granulosum of the keratinized epidermis; causes 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 subcutaneus tissues; associated with furuncolosis
Superantigens / pyrogenic exotoxins		Stimulate T-cells non-specifically to cytokine release
Enterotoxins A, B,C, D, E, G, H, K (and others)	*sea, seb, sec, etcetera…*	Cause staphylococcal food poisoning and half of the cases of non-menstrual toxic shock syndrome (TSS)
Toxic shock syndrome toxin	TSST-1 / *tst*	Responsible for 75% of cases of TSS (all cases of menstrual TSS)

Table 1C: Microbial surface proteins 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

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, small, soluble proteins that enable it to suppress the innate immunity and survive in the human body.

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.[8] Furthermore, modification of cell wall teichoic acids promotes *S. aureus* resistance to AMPs.[9]

Complement evasion

The complement cascade serves three major functions in innate immunity, that is (1) to opsonize bacteria (through C3b, figure 2), (2) to attract phagocytes (through C3a and C5a), and (3) to perturb bacterial membranes of Gram-negative bacteria (C5b–9, the Membrane Attack Complex).[10,11] Complement activation is initiated by three different pathways (classical, lectin or alternative) that result in the formation of C3 convertases, bimolecular enzymes that cleave the central complement protein C3 (Figure 3). 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 chemo-attractant) and C5b (part of the membrane attack complex). *S. aureus* produces a variety of molecules that interfere with the complement cascade:

-The secreted Staphylococcal complement inhibitor (SCIN) blocks C3 convertases through stabilization of these complexes.[12]

-Extracellular fibrinogen binding protein (Efb) and Extracellular complement binding protein (Ecb) target surface-bound C3b containing convertases,[13] inhibiting C3b deposition via the alternative pathway and C5a formation via all pathways.

-The staphylococcal cell wall-associated protein Sbi has two extracellular complement-inhibitory domains that bind C3.[30]

-Staphylococcal superantigen-like (SSL) protein 7 specifically binds to C5, preventing cleavage by C5 convertases and subsequent formation of C5a.[14]

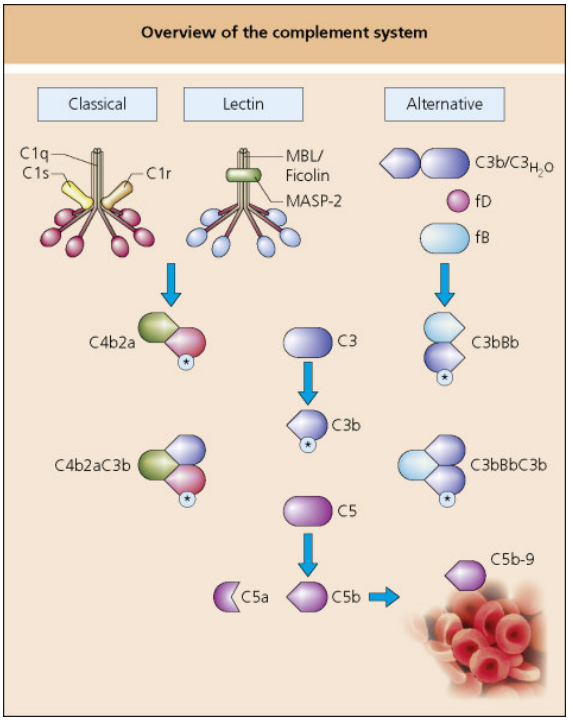


Figure 3: Schematic overview of the complement system.

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. (The red blood cells supposedly represent bacteria).

Inhibition of neutrophil recruitment

Effective eradication of *S. aureus* depends on phagocytosis and intracellular killing by cells of the monocyte and granulocyte lineage, mainly neutrophils.[15] This critical role is reflected by the increased risk for *S. aureus* infections in patients with defects in granulocyte function, both inherited defects (e.g., chronic granulomatous disease, myeloperoxidase deficiency, leucocyte adhesion deficiencies) and acquired defects (such as diabetes mellitus rheumatoid arthritis, and HIV). During an infection, neutrophils are rapidly recruited from circulation to sites of microbial invasion by host stimuli (complement fragments C3a and C5a, IL-8, Leukotriene B4) and pathogen-derived stimuli (fMLP, phenol soluble modulins).[16] 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.[17] Subsequently, the neutrophils migrate through the endothelial cell layer (diapedesis) and move towards the site of infection under a gradient of chemo-attractant substances, the foremost being C5a (chemotaxis).

The Staphylococcal Superantigen Like 5 (SSL5) inhibits the interaction between P-selectin on endothelial cells and P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils, thereby inhibiting neutrophil rolling on the endothelium.[18] Extracellular adherence protein (Eap) of *S. aureus* inhibits the next steps in neutrophil extravasion by its interactions with the intercellular adhesion molecule 1 (ICAM-1), fibrinogen or vitronectin.[19] Finally, *S. aureus* secretes two molecules that specifically block chemotactic movement of phagocytes: the Chemotaxis inhibitory protein of *S. aureus* (CHIPS) which binds the formylated peptide receptor and the C5a receptor,[20] and the FPRL1 inhibitory protein (FLIPr) which binds the formylated peptide receptor like-1.[21]

Resistance to phagocytosis and intracellular killing

Neutrophil ingestion of opsonized micro-organisms in phagosomes depends on the interaction between opsonic ligands on the bacterium and receptors on the phagocyte membrane. Upon bacterial uptake, the interaction of opsonic ligands with receptors triggers

the release of oxidative products and granule contents (e.g. myeloperoxide, defensins) into the phagosome destroying the ingested particle. *S. aureus* is most efficiently phagocytosed after opsonization by both complement and antibodies.

Staphylococci are often surrounded by a loose-fitting polysaccharide capsule that interferes with opsonization, either by shielding the cell-wall from reacting with antibodies and complement, or by hindering the binding of surface-bound complement factors to phagocyte receptors. Effective opsonization of encapsulated *S. aureus* therefore requires anticapsular antibodies, largely directed against the O-acetyl group of the capsular polysaccharide. Eleven putative capsular serotypes have been proposed; expression of type 5 and 8 capsules (75% of all capsulated strains) is associated with increased virulence in animal infection models.[22]

Staphylokinase (SAK), a plasminogen-activating molecule, generates surface-bound plasmin. This, in turn, cleaves the major opsonins IgG and C3b preventing their recognition by phagocytes. The *S. aureus* surface protein A (SpA) binds the Fc terminal of human IgG, covering the bacterial surface with outward-facing IgG molecules that cannot be recognized by Fc-receptors on phagocytes. SSL7 interacts with human IgA1 and IgA2 to interfere with IgA-dependent cellular effector functions.[23]

Once phagocytosed, staphylococci may inhibit killing and travel through the bloodstream within neutrophils. The golden pigment (for which *S. aureus* is named) is a carotenoid molecule with antioxidant properties that scavenges free oxygen radicals.[24] Furthermore, *S. aureus* can resist oxidative stress by two superoxide dismutase enzymes that remove superoxide.

Cytolytic toxins

Cytotoxins secreted by *S. aureus* lyse host cells by forming β-barrel pores in cytoplasmic membranes of target cells. Well-known examples are α-toxin and the bi-component leukotoxins γ-toxin and the exfoliative toxins (ETA and ETB), that cause separation of the dermis at the granular cell layer resulting in extensive scalding (staphylococcal scalded syndrome).

Panton-Valentine Leukocidin (PVL) is a cytotoxin associated with furuncolosis and hemorrhagic pneumonia.[25] In the last decade community-acquired infections, mainly skin and soft tissue infections, are increasingly caused by PVL-positive community-acquired MRSA strains (CA-MRSA). Although PVL is found in 69 to 98% of CA-MRSA clinical isolates,[26] the exact role of PVL in the pathogenesis of these infections is not completely clear and studies of its pathogenicity in animal models have yielded conflicting results.

Phenol soluble modulins are a class of secreted staphylococcal peptides that recruit, activate and subsequently lyse human neutrophils, thus eliminating the main cellular defense against *S. aureus* infection. A recent study revealed a contribution of these factors to the virulence of CA-MRSA.[16]

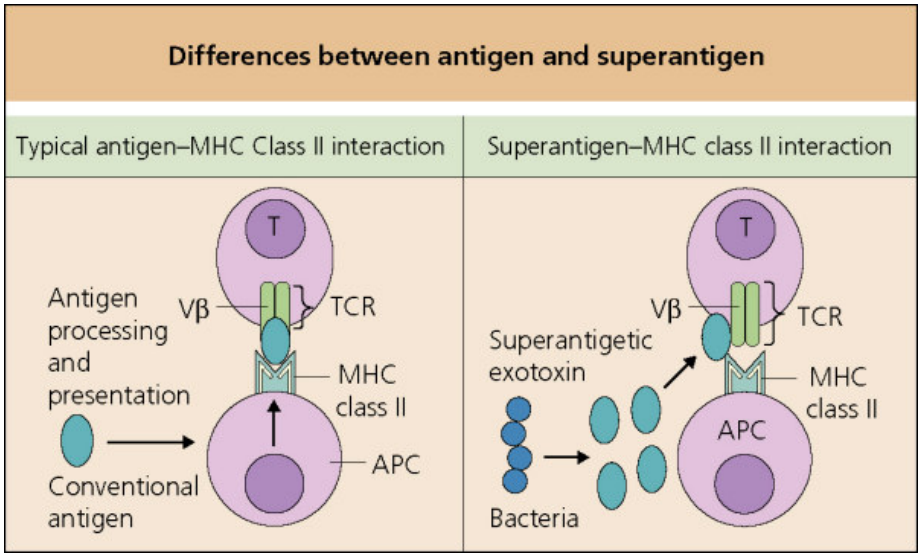


Figure 4: Differences between antigen and superantigen.

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 & Franz-Josef Schmitz**.**

Immuno stimulatory molecules

Superantigens (or pyrogenic exotoxins) are the agents responsible for toxic shock syndrome (TSS) (Figure 4). These extracelullar proteins bind to the exterior surface of MHC 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. Up to a third of the T-cells may be stimulated to proliferate and release cytokines. Due to this non-specific activation of T-cells, the immune response against superantigenic toxins is impaired and patients suffering TSS often lack specific antibodies against superantigens.[27]

Toxic shock syndrome toxin (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 enterotoxins may also cause gastro-intestinal disease (*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. Staphylococcal protein A (SpA) binds to the surface of B lymphocytes, where it also exerts a potent immunostimulatory activity.[28]

Interactions with the coagulation system

S. aureus produces extracellular coagulase which binds to prothrombin to form a complex called staphylothrombin, hereby activating the protease activity of thrombin. The activated trombin converts fibrinogen to fibrin, causing localized clotting and shielding the bacteria from host defenses.[29] In addition, most strains express a fibrin/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 insertion sequences, bacteriophages, plasmids, transposons and pathogenicity islands. The genes for Exfoliative toxins A and B are located on a bacteriophage and a plasmid respectively, and have been demonstrated in 0-2% of

strains. PVL is located on a bacteriophage and was present in only 2% of isolates. The pathogenicity island harboring toxic shock syndrome toxin 1 (TSST-1) is found in 14-24%. The immune modulators CHIPS, SCIN, SAK and SEA are clustered together on a β-hemolysin converting bacteriophage present in 90% of clinical *S. aureus* isolates.[20]

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, through a positive feedback loop, switches from the preferential expression of surface adhesins during the exponential phase of growth, to the expression of exoproteins during post-exponential and stationary growth phases.[30] When bacterial density increases above a certain level, *agr* drives the transcription of RNAIII, an RNA-molecule which modulates virulence factor expression both at the transcriptional and translational levels. *Agr*-downregulated gene products include protein A (*spa*) and fibronectin-binding protein (*fnb*); the hemolysins, enterotoxins, exfoliatins and PSMs are examples of virulence factors which are upregulated by *agr*. Most other currently known regulatory mechanisms interact at some level with *agr*, either synergistically or reciprocally.[31]

Epidemiology and clinical presentation

Epidemiology

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). Generally, the established flora of the nose prevents the acquisition of new strains. Person to person spread is believed to occur mainly by direct hand/skin-contact, in a hospital setting 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 in rest, they are surrounded by

0.01 to 0.1 colony-forming units / m^3, but up to 0.3 CFU/m3 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. A number of outbreaks have been attributed to single airborne spreaders.[34] Although acquisition occurs primarily on the skin, *S. aureus* can only persist on the long-term if the nares or perineum become colonized.

Cross-sectional prevalence rates of colonization among healthy subjects differ extensively; from as low as 14% in African American job applicants, to as high as 64% in British hospital personnel.[35] Persons can be subdivided into "persistent carriers", "intermittent carriers", and "non-carriers", based upon the proportion of positive nasal swabs as well as the quantity of isolates. Based on different definitions and different populations of healthy individuals, 2-71% are considered non-carriers, 19-70% are considered intermittent carriers and 9-37% are considered persistent carriers.[35] Groups with documented increased risks of colonization include patients with type 1 diabetes, patients undergoing hemodialysis, surgical patients, intravenous drug users, and HIV-patients. Heavy antibiotic pressure may lower (detectable) colonization rates.

Nasal carriage of *S. aureus* is a risk factor for subsequent infection. 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 without *S. aureus* carriage pre-operatively. A relation between colonization and (bacteremic) infection has also been demonstrated for CAPD-patients, hemodialysis patients, HIV-patients, and hospitalized patients colonized with MRSA. Infection risk was even higher in patients who had both nasal and rectal *S. aureus* colonization than in those who only had nasal colonization, suggesting a relation between the bacterial load and the chance of subsequent infection.[36] When linking *S. aureus* isolates from blood and previously obtained specimens from the anterior nares of 219 patients with *S. aureus* bacteremia, genotypical identity was demonstrated in 180 of 219 patients (82.2 %).[37] In another study patients colonized with *S. aureus* in the nares upon hospital admission and subsequently developing nosocomial *S. aureus* bacteremia (with identical strains) had a lower mortality than those developing *S. aureus* bacteremia while not

already being colonized when admitted.[38] It was suggested that colonization may offer protection against an overexagerated inflammatory response during infection.

S. aureus has been reported to persist in dust and on fomites for up to 7 months. It has been isolated from door handles, desk tops, water bowls, computer keyboards, faucet handles, and blood pressure cuffs. Although (methicillin resistant) *S. aureus* was isolated from inanimate surfaces in several documented outbreaks, the role of environmental contamination or airborne transmission is controversial.

Clinical presentation

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 furuncolosis, but also pneumonia (typically post-influenza), osteomyelitis and acute endocarditis. Staphylococcal toxins may be responsible for food-poisoning, staphylococcal toxic shock syndrome (TSS) and staphylococcal scalded skin syndrome (SSSS).[39]

In nosocomial settings *S. aureus* is the main causative agent of post-operative 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 blood stream infections, hospital acquired pneumonia and ventilator-associated pneumonia. *S. aureus* bacteremia (SAB), although rather 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.[40]

S. aureus infrequently causes urinary tract infections, predominantly in patients with recent urinary tract surgery or other manipulations, and in patients with urinary tract obstruction.[41]

Epidemiology of antibiotic resistance

Although penicillin was initially considered a miracle drug for *S. aureus*, the first cases of penicillin-resistance, due to β-lactamase production, were already reported in as early as 1944. By 1950 approximately 80% of hospital-acquired infections were caused by these penicillinase producers. Shortly after the introduction of the β-lactamase-stable antibiotics (such as methicillin), in the early 1960s, the first reports of methicillin-resistant *S. aureus* (MRSA) appeared. Methicillin-resistance resulted from the production of an alternative penicillin-binding protein, PBP 2A (or PBP2'), encoded by the mecA-gene on the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element.[42]

In most countries colonization with MRSA is now endemic within hospital populations. In the USA, for instance, the proportion of MRSA among nosocomial *S. aureus* bacteremia increased from 2.4% in 1975 to 29% in 1991, and in American intensive care units the proportion of MRSA had risen to nearly 60% in 2003.[43] A few countries, such as the Netherlands and the Scandinavian countries, have succeeded in containing the nosocomial spread of MRSA by using extensive infection control measures and restrictive antibiotic policies. As a result of the Dutch "search-and-destroy"-policy for MRSA, the prevalence of MRSA-colonization in the Dutch population is still under 0.1% (data from 2000).[32]

An epidemiological characteristic of MRSA has been the almost complete absence of patient to patient transmission outside the hospital. Recently, though, MRSA strains with clear potential for transmission between healthy subjects have emerged. The most prominent example of these so-called community-acquired MRSA (CA-MRSA) is the USA300 genotype (based on pulsed-field gel electrophoresis analysis).[44] Outbreaks have occurred in communities of men who have sex with men, homeless populations, inmates in correctional facilities, military recruits, sports teams and children in day-care centres. Recently, percentages of CA-MRSA in community-acquired *S. aureus* infections have been reported as high as 76% by different American healthcare institutions. In contrast to HA-MRSA, young age and absence of comorbidity are associated with CA-MRSA infections.[45] Apparently, USA300 has acquired a number mobile genetic elements that encode resistance and

virulence determinants that could enhance fitness and pathogenicity. In several European countries, MRSA appears also to be widely spread among animals, such as pigs and calves, with subsequent transmission of MRSA to caretakers.

Based upon multilocus sequence typing (MLST) the population structure of MRSA is characterized by five major clonal complexes (CC's): CC5, CC8, CC22, CC30 and CC45. Within these five clonal complexes different SCCmec-types are found, indicating that MRSA-clones emerged by multiple independent introductions of the mecA-gene.[46] Four of the five major CCs represent pandemic clones of HA-MRSA: CC5 (New York / Japan-clone, pediatric clone), CC8 (Vienna-clone, Brazilian, Portuguese, Irish-1 and Iberian clone), CC22 (Barnim-clone) and CC45 (Berlin clone). USA300 belongs to CC8. The non-typeable MRSA linked to the animal reservoir in Europe is ST398, which is not phylogenetically linked to any of the major MRSA CCs.

The high prevalence of MRSA in hospitals necessitated extensive use of the glycopeptide vancomycin, which has been the "last resort" antibiotic for MRSA infections for many years. The first *S. aureus* strain with reduced vancomycin susceptibility was isolated in Japan in 1997 (the Mu50 strain) and has now emerged among all five major hospital MRSA lineages.[47] In 2005 0.2% of 240.000 *S. aureus* isolates in the Surveillance Network data from US laboratories were vancomycin intermediate resistant *S. aureus* (VISA, defined by a MIC >2 and ≤ 8 mg/l).[48] Intermediate resistance to glycopeptides is associated with thickening of the bacterial cell wall, but the exact mechanism remains to be elucidated. Unfortunately, reduced susceptibility to vancomycin appears to be associated with reduced susceptibility to new drugs such as linezolid and daptomycin.[49]

High-level VRSA (MIC ≥32 mg/l) can result from acquisition of the enterococcal VanA resistance gene by *S. aureus*.[50] As of September 2007, seven cases of vanA-mediated VRSA have been reported, all from the US and five of them from Michigan.

Prevention

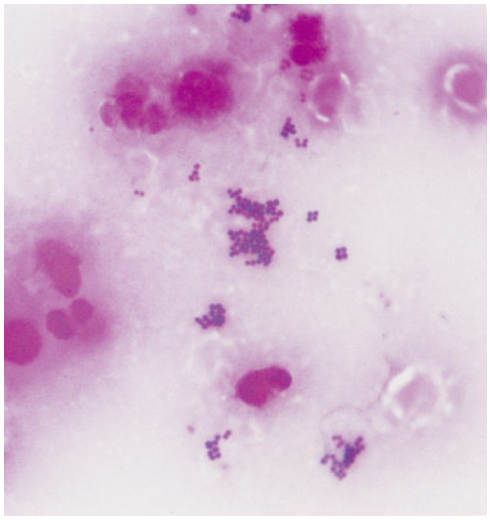
Prevention of MRSA/spread

Several countries have implemented nationwide "search-and-destroy policies" to limit the spread of MRSA within hospital settings. These strategies were implemented in the late nineteen eighties, when 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, the admitted patients with an increased risk of MRSA-carriage are screened and precautiously isolated, until culture results rule out MRSA-carriage. Finally, contact patients and health care workers are screened for MRSA-carriage in case of unexpected detection of MRSA in a hospitalized patient.

Decolonization

Eradication of *S. aureus* carriage pre-operatively has been evaluated in several studies. The use of oral rifampicin, which appeared effective in a cohort of hemodialysis patients, has been largely abandoned, due to increasing rifampicin resistance and associated toxic effects of this agent. Mupirocin is highly effective in short-term nasal eradication of carriers (87-94% patients are negative after 1 week), but high recurrence rates after six months were found in one study. Two randomized placebo-controlled trials with 4030 and 614 surgical patients failed to demonstrate a statistically significant reduction in post-operative surgical site infections when using decolonization with mupuricin. However, in the largest of these trials, there was a significant difference in nosocomial *S. aureus* infections observed, among *S. aureus* carriers (4% vs 7.7%).[53,54] In smaller populations, such as CAPD-patients, hemodialysis patients and patients with recurrent skin infections mupirocin treatment was associated with significant reductions of *S. aureus* infections.[55]

Figure 5 (right): *Staphylococcus aureus* in a Gram stain of pus. Courtesy of Jan Verhoef, Ad Fluit & Franz-Josef Schmitz**.**



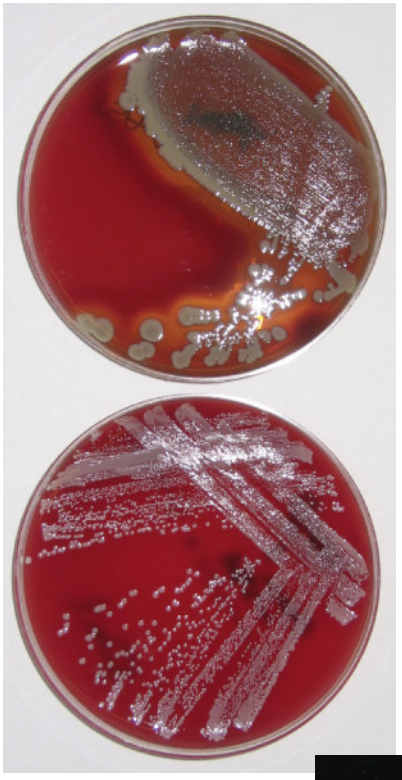
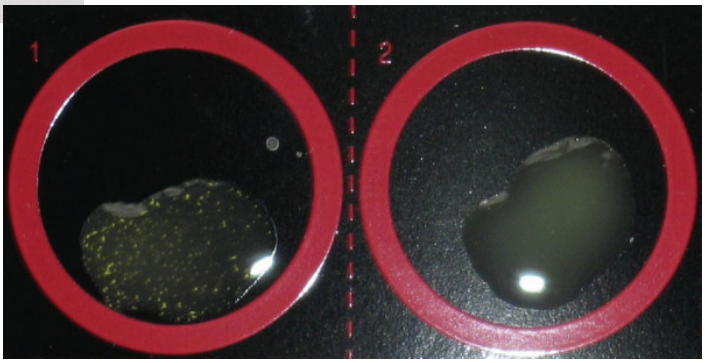


Figure 6 (left) : Growth of *Staphylococcus aureus* (upper) and *Staphylococcus epidermidis* (lower) on trypticase soy agar with sheep blood.

Figure 7 (below): Slide coagulase test.
Latex particles coated with coated with fibrinogen and IgG agglutinate when a colony of *Staphylococcus aureus* is suspended in the solution (1), and negative control (2).



Diagnostic Microbiology

Isolation and determination

Most staphylococcal lesions contain numerous PMNLs and large numbers of *S. aureus*, which may readily be demonstrated by a direct Gram smear of pus (Figure 5). Direct Gram smears of sputum samples may also assist in rapid identification of staphylococcal pneumonia.

Staphylococci generally grow overnight on most conventional bacteriological media. The preferential medium for isolation is (sheep) blood agar, on which they form colonies of 2 mm or more in diameter (Figure 6). Blood cultures from untreated bacteremia patients are usually positive after overnight incubation. Staphylococci may grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Differentiation from other Gram-positive cocci may be aided by the determination of a couple of characteristics (Table 2). The fermentation of mannitol by *S. aureus* is used in mannitol salt agar to screen for this bacterium in clinical or environmental samples.[56]

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 7), testing for free coagulase, and for DNAse / thermostable endonuclease. However, non-optimal sensitivity of these tests has been reported, especially in identifying MRSA. 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.

S. aureus can also be identified by molecular techniques demonstrating specific genes such as nuclease (*nuc*), coagulase (*coa*), protein A (*spa*), surface-associated fibrinogen-binding protein or the sa442 gene; yet, none of these techniques are 100% sensitive or specific.

Table 2: Differentiation of Staphylococci from other Gram-positive cocci

	Staphylococcus spp	Micrococcus spp	*Kocuria kristinae*	*Rothia mucilaginosa*
Gram-stain	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	Creme-coloured to yellow / white	Creme-coloured to canary yellow	Creme-coloured to canary yellow	Clear to white
Mupirocin	Susceptible	Resistant	Resistant	-
Bacitracin	Resistant	Susceptible	Susceptible	Susceptible
Growth in 6,5% NaCl	Yes	Yes	Yes	No
Oxidase	Negative	Positive	Positive	Negative
Catalase	Positive	Positive	Positive	Weakly positive or negative

Susceptibility testing

S. aureus susceptibility testing can be performed by disc diffusion or E-test on standard bacteriological media, or by micro and macro broth dilutions. Guidelines and breakpoints are available from the Clinical and Laboratory Standards Institute (CLSI), the European committee on Antibiotic Susceptibility Testing (EUCAST), and the International Organization for Standardization (ISO). Automated systems are available for broth dilution susceptibility testing. Although adequate for most antibiotics, certain considerations apply.

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).[57] The methylase genes are associated with clinical failure of clindamycin therapy, but as clindamycin does not induce expression of these genes in vitro, tested strains may wrongly appear susceptible to the antibiotic. Erythromycin *is* a potent inducer of methylases in vitro, and erythromycin-resistance is an initial clue to MLS_B–resistance. An induction test (with an erythromycin and clindamycin

disk placed 20-26 mm from each other) should, therefore, be performed on erythromycin-resistant *S. aureus* strains. After overnight incubation, MLS_B–resistance will be induced by erythromycin, creating a characteristic D-shaped inhibition zone around the clindamycin disk. Such strains should also be considered resistant to the streptogramin B antibiotic quinopristin-dalfopristin. Enzymatic inactivation of clindamycin is another potential resistance mechanism, but this occurs much less frequently.

Isoxazolyl-penicillins

Identifying methicillin-resistant strains in the bacteriological laboratory is challenging. The gold standard for identification of MRSA is the detection of the MecA-gene, which encodes the altered penicillin-binding protein PBP2a. Although all MRSA-strains harbor the MecA-gene, in heterogenous MRSA populations expression of PBP2a is suppressed in most colony forming units and may not always be detected by disk diffusion with oxacillin 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.[58] Rapid latex agglutination assays, which react to PBP2A may confirm MRSA, but these tests are less sensitive than cefoxitin-screening.[58]

Screening for MRSA-colonization is performed on selective media (both liquid and solid media), which contain either oxacillin or cefoxitin. Several rapid MRSA detection media are available (both comercially and in-house produced media), 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%.[59]

Recently, rapid molecular detection tests for MRSA-screening have been introduced. PCR targets are usually the MecA-gene in combination with a specific *S. aureus* gene (e.g. the sa442-gene, the coagulase-gene or the nuclease-gene). PCR-based techniques detect MRSA in screening specimens within 4-6 hours and appear to be highly specific and sensitive,[60] but performance may vary: depending on the primerset used, certain MRSA-clones will not be detected. For the moment, cultures remain essential in MRSA-screening, especially to confirm positive PCR-results.

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. The currently most widely employed techniques are discussed below.

Pulsed Field Gel Electrophoresis (PFGE)

PGFE is based on the digestion of bacterial DNA with restriction endonucleases (for MRSA usually *smaI*), generating large fragments of DNA (10-800 Kb). A sharper resolution of the fragments is obtained by alternating the direction of the electric current on the separation gel. PFGE has a high discriminatory power and the results are highly reproducible;[61] the technique has therefore been proposed as the gold standard for MRSA typing. However, there are limitations to its use, such as the long time interval until the the final results are obtained, and the cost of reagents and specialised equipment.[61] Furthermore, even though relatively few bands are generated, small differences in electrophoresis conditions can alter the distance travelled by each band, and complicate the comparison between isolates submitted to electrophoresis in different gels.

Multi Locus Sequence Typing (MLST)

MLST characterizes bacterial isolates by using the sequences of internal fragments of seven housekeeping genes.[62] Every polymorphism in 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. Advantage of MLST include its unambiguous nomenclature, easy global exchange of typing data and the possibility for population structure and evolutionary analyses. Genetically related ST's can be clustered by MLST into so-called clonal complexes (CC's). On the downside, MLST is less discriminatory than PFGE and quite expensive. Websites are available where MLST-results are collected for a number of different bacteria (www.mlst.net).

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.[63] Spa-typing is highly reproducible and easy to interpret. It has has less discriminatory power than MLST and PFGE, but is also less costly and easier to perform. A web-based reference database (www.spaserver.ridom.de), which uses a standardized spa-type nomenclature, permits a global epidemiological comparison of isolated MRSA-strains.

Management of *S. aureus* infections

Management of *S. aureus* infections often involves the combined use 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), sometimes in combination with topical antibiotics (mupirocin, fusidic acid) or even systemic antibiotics.

Beta-lactam antibiotics are the agents of first choice in the treatment of (severe) systemic MSSA-infections. Comparative studies between different beta-lactam antibiotics are lacking, as are studies evaluated different lengths of treatment. Isoxazolyl-penicillins (cloxacillin, dicloxacillin, flucloxacillin), penicillin / betalactamase-inhibitor combinations (amoxicillin/clavulanic acid, piperacillin/tazobactam), first and second generation cephalosporins and carbapenems are considered equally effective in the treatment of MSSA infections. The clinical experience with the isoxazolyl-penicillins and their narrow spectrum of activity makes them the first choice of therapy. Vancomycin, a glycopeptide, is the antibiotic of choice for (severe) systemic infections with MRSA and in patients with beta-lactam allergy. The glycopeptides are, however, significantly less active than the beta-lactams.[64] CA-MRSA strain are mostly still susceptible to several antibiotic classes and can often be treated with co-trimoxazole or clindamycin (used especially in the treatment of

abscesses, for its high tissue-penetration). Other antibiotics with anti-staphylococcal activity include the glycopeptide teicoplanin, the macrolides, the newer classes of the fluoroquinolones, rifampicin, fusidic acid, tigecycline, linezolid and daptomycin.

Because of the severe complications of *S. aureus* bacteremia and its propensity to relaps,[40] treatment with systemic antibiotic therapy for a minimum of two weeks is recommended.[65] In vitro, aminoglycosides act synergistically in *S. aureus* killing, and addition of an aminoglycoside (most often gentamicin) may shorten the duration of fever and bacteremia, although improved outcome with this combined therapy has not been demonstrated. In any case, because of its nephrotoxic side-effects, it is advised to limit the duration of aminoglycoside-therapy to 3 to 5 days. Because of the high a priori risk of endocarditis in patients with *S. aureus* bacteremia (12% in a large observational study),[40] trans-esophageal echocardiography should always be considered in these patients, and it is mandatory in all patients who fail to improve or maintain positive blood cultures under adequate antibiotic therapy.[66]

Intravascular catheters colonized with *S. aureus* are associated with a high risk of subsequent *S. aureus* bacteremic complications, even when there were no signs of catheter site infection at the time of line removal or negative blood cultures. Anti-staphylococcal antibiotic therapy in such cases was associated with markedly reduced incidence of complications and is, therefore, advised.[67]

Recently, several new anti-staphylococcal drugs (linezolid, quinopristin/dalfopristin and daptomycin) have been approved. Telavancin and dalbavancin, two lipoglycopeptides, are currently in the registration process, and several cephalosporins and carbapenems with anti-MRSA activity are in phase III studies. Since clinical trials evaluating new drugs are usually manufacturer-sponsored studies intented for registration, designed as non-inferiority studies, and underpowered to demonstrate superiority of new drugs when compared to standard therapy. Thus far, in randomized controlled trials, none of the new agents has been proven superior to standard therapy in regard to mortality and clinical cure, although trends towards superiority are sometimes observed, and meta-analysis may yield significant differences. At the moment, there is clearly no such thing as the “ideal anti-MRSA

antibiotic": Prolonged therapy with linezolid (one of the few new anti-MRSA drugs available in an oral formulation) may cause central (optic) neuropathy and bone marrow depression (especially thrombocytopenia), and it is therefore advised not to extend therapy beyond 28 days. Daptomycin is inhibited by pulmonary surfactant, and should not be used in case of a clinical suspicion of pneumonia.[68]

One thing which *has* become clear from all the clinical trials conducted to evaluate new antibiotics, is that intravenous administration of vancomycin, the predominant comparator antibiotic for new anti-MRSA agents, is a safe with limited, if any, nephrotoxic effects. The nephrotoxicity formerly attributed to vancomycin probably resulted from either contamination of previously used vancomycin-formulations (these suspensions were known as Mississippi mud for their turbid, dark brown appearance), the underlying illness of patients, or co-medication.[69] Vancomycin is believed to enhance aminoglycoside nephrotoxicity but the evidence for this effect is limited and contradictory.[70] Nevertheless, it seems prudent to avoid the combination of these drugs as much as possible, especially when longer treatment periods are indicated. Vancomycin trough levels should be monitored to ensure adequate (high enough) dosing in patients with severe infections, especially when patients fail to respond to treatment.

Rifampicin and fusidic acid, both available in oral and parenteral formulations, are regularly used in combination therapy for particular *S. aureus* infections. Both agents are less suitable as monotherapy, due to the rapid development of resistance by *S. aureus* under these regimens. Because of its penetration in biofilms and its activity on slowly dividing bacteria, rifampicin is often part of an antibiotic regimen to treat of foreign body infections (e.g. prosthetic joints) and endocarditis, in particular endocarditis involving prosthetic heart valves,[71] Fusidic acid is used in combination-therapy for the treatment of MRSA-infections and in *S. aureus* endocarditis.[71] Unfortunately, clinical evidence regarding the use of these two drugs remains scarce, and further studies are needed to determine their exact place in clinical practice.

References

1. Wieser M. Emended descriptions of the genus Micrococcus, *Micrococcus luteus* (Cohn 1872) and *Micrococcus lylae* (Kloos et al. 1974). **Int J Syst Evol Microbiol 2002**; 52:629-637.
2. Liu H, Xu Y, Ma Y, Zhou P. Characterization of *Micrococcus antarcticus* sp. nov., a psychrophilic bacterium from Antartica. **Int J Syst Evol Microbiol 2000**; 50:715-719.
3. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification system, Actinobacteria classi s nov. **Int J Syst Bacteriol 1997**; 47:479-491.
4. Ogston A. Report upon microorganisms in surgical diseases. **Britisch Medical Journal 1881**; 1:369-375.
5. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. **J Infect Dis. 2008**; 198:962-70
6. Westerlund B, Korhonen TK. Bacterial proteins binding to the mammalian extracellular matrix. **Mol Microbiol 1993**; 9:687-694.
7. Bowden MG, Heuck AP, Ponnuraj K, Kolosova E, Choe D, Gurusiddappa S 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**; 283:638-647.
8. 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**; 172:1169-1176.
9. Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Götz F. Inactivation of the dlt operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. **J Biol Chem 1999**; 274:8405-8410.
10. Walport MJ. Complement. Second of two parts. **N Engl J Med 2001**; 344:1140-1144.
11. Walport MJ. Complement. First of two parts. **N Engl J Med 2001**; 344:1058-1066.
12. Rooijakkers SH, Milder FJ, Bardoel BW, Ruyken M, van Strijp JA, Gros P. Staphylococcal complement inhibitor: structure and active sites. **J Immunol 2007**; 179:2989-2998.

13. Jongerius I, Köhl J, Pandey MK, Ruyken M, Van Kessel KP, van Strijp JA et al. Staphylococcal complement evasion by various convertase-blocking molecules. **J Exp Med 2007**; 204:2461-2471.

14. 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**; 174:2926-2633.

15. Foster TJ. Immune evasion by staphylococci. **Nat Rev Microbiol 2005**; 3:948-958.

16. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. **Nat Med 2007**; 13:1510-1514.

17. Ley K. Integration of inflammatory signals by rolling neutrophils. **Immunol Rev 2002**; 186:18.

18. Bestebroer J, Poppelier MJ, Ulfman LH, Lenting PJ, Denis CV, Van Kessel KP et al. Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. **Blood 2007**; 109:2936-2943.

19. Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, Flock JI et al. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. **Nat Med 2002**; 8:687-693.

20. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC et al. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. **J Exp Med 2004**; 199:687-695.

21. Prat C, Bestebroer J, de Haas CJ, van Strijp JA, Van Kessel KP. A new staphylococcal anti-inflammatory protein that antagonizes the formyl peptide receptor-like 1. **J Immunol 2006**; 177:8017-8026.

22. O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. **Clin Microbiol Rev 2004**; 17:218-234.

23. Wines BD, Willoughby N, Fraser JD, Hogarth PM. A competitive mechanism for staphylococcal toxin SSL7 inhibiting the leukocyte IgA receptor, Fc alphaRI, is revealed by SSL7 binding at the C alpha2/C alpha3 interface of IgA. **J Biol Chem 2006**; 281:1389-1393.

24. Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF et al. Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. **J Exp Med 2005**; 202:209-215.

25. Foster TJ. Immune evasion by staphylococci. **Nat Rev Microbiol 2005**; 3:948-958.

26. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB et al. Methicillin-resistant S. aureus infections among patients in the emergency department. **N Engl J Med 2006**; 355:666-674.

27. Foster TJ. Immune evasion by staphylococci. **Nat Rev Microbiol 2005**; 3:948-958.

28. Graille M, Stura EA, Corper AL, Sutton BJ, Taussig MJ, Charbonnier JB et al. Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. **Proc Natl Acad Sci USA 2000**; 97:5399-5404.

29. 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**; 261:1427-1433.

30. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. **Mol Microbiol 2003**; 48:1429-1449.

31. Bronner S, Monteil H, Prévost G. Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. **FEMS Microbiol Rev 2004**; 28:183-200.

32. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CM, Meester MH, et al: Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. **J Hosp Infect. 2004**; 56:321-325.

33. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. **Lancet Infect Dis 2002**; 2:677-685.

34. Sherertz RJ, Bassetti S, Bassetti-Wyss B. "Cloud" health-care workers. **Emerg Infect Dis 2001**; 2:677-685.

35. Vandenbergh MF, Yzerman.E.P., van Belkum A, Boelens HA, Sijmons M, Verbrugh HA. Follow-up of *Staphylococcus aureus* nasal carriage after 8 years: redefining the persistent carrier state. **J Clin Microbiol 1999**; 37:3133-3140.

36. Squier C, Rihs JD, Risa K.J., Sagnimeni A, Wagener MM, Stout J. *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**; 23:495-501.

37. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. **N Engl J Med 2001**; 344:11-16.

38. Wertheim HF, Vos MC, Ott A, Van Belkum PA, Voss PA, Kluytmans JA et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. **Lancet 2004**; 364:703-705.

39. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. **Clin Microbiol Rev 2000**; 13:16-34.

40. Fowler VG, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. **Arch Intern Med 2003**; 163:2066-2022.

41. Muder RR, Brennen C, Rins JD, Wagener MM, Obman A, Stout JE 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**; 42:46-50.

42. Hanssen AM, Ericsson Sollid JU. SCCmec in staphylococci: genes on the move. **FEMS Immunol Med Microbiol 2006**; 46:8-20.

43. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. **Am J Infect Control 2004**; 32:470-485.

44. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. **Ann Intern Med 2006**; 144:309-317.

45. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud.D.J., Etienne J et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. **JAMA 2003**; 290:2976-2984.

46. Enright MC, Robinson DA, Randle G, Feil E, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). **Proc Natl Acad Sci USA 2002**; 99:7687-7692.

47. Howe RA, Monk A, Wootton M, Walsh TR, Enright MC. Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. **Emerg Infect Dis 2004**; 10:855-857.

48. Tenover FC, Moellering RCj. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. **Clin Infect Dis 2007**; 44:1208-1215.

49. Cui L, Tominaga E, Neoh HM, Hiramatsu K. Correlation between Reduced Daptomycin Susceptibility and Vancomycin Resistance in Vancomycin-Intermediate Staphylococcus aureus. **Antimicrob Agents Chemother 2006**; 50:1079-1082.

50. Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. **N Engl J Med 2003**; 348:1342-1347.

51. Chu VH, Woods CW, Miro JM, Hoen B, Cabell CH, Pappas PA et al. Emergence of coagulase-negative staphylococci as a cause of native valve endocarditis. **Clin Infect Dis 2008**; 46:232-242.

52. Nouwen JL, van Belkum A, de Maris S, Sluijs J, Wielenga JJ, Kluytmans JA et al. Clonal expansion of *Staphylococcus epidermidis* strains causing Hickman catheter-related infections in a hemato-oncologic department. **J Clin Microbiol 1998**; 36:2696-2702.

53. Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. **N Engl J Med 2002**; 346:1871-1877.

54. Kalmeijer MD, Coertjens H, van Nieuwland-Bollen PM, Bogaers-Hofman D, de Baere GA, Stuurman A et al. Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. **Clin Infect Dis 2002**; 35:353-358.

55. Raz R, Miron D, Colodner R, Staler Z, Samara Z, Keness Y. A 1-year trial of nasal mupirocin in the prevention of recurrent staphylococcal nasal colonization and skin infection. **Arch Intern Med 1996**; 156:1109-1112.

56. Blair EB, Emerson JS, Tull AH. A new medium, salt mannitol plasma agar, for the isolation of *Staphylococcus aureus*. **Am J Clin Pathol 1967**; 47:30-39.

57. 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**; 43:2823-2830.

58. 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**; 40:2766-2771.

59. 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**; 12:1168-1174.

60. Huletsky A, Lebel P, Picard FJ, Bernier M, Gagnon M, Boucher N et al. Identification of methicillin-resistant Staphylococcus aureus carriage in less than 1 hour during a hospital surveillance program. **Clin Infect Dis 2005**; 40:976-981.

61. Weller TM. Methicillin-resistant *Staphylococcus aureus* typing methods: which should be the international standard? **J Hos Infect 2000**; 44:160-172.

62. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. **Proc Natl Acad Sci USA 1998**; 95:3140-3145.

63. Frénay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J et al. Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. **Eur J Clin Microbiol Infect Dis 1996**; 15:60-64.

64. Fortún J, Pérez-Molina JA, Añón MT, Martínez-Beltrán J, Loza E, Guerrero A. Right-sided endocarditis caused by *Staphylococcus aureus* in drug abusers. **Antimicrob Agents Chemother 1995**; 39:525-528.

65. Mermel LA, Farr BM, Sherertz RJ, Raad II, O'Grady N, Harris JS et al. Guidelines for the management of intravascular catheter-related infections. **Clin Infect Dis 2001**; 32:1249-1272.

66. Mermel LA, Farr BM, Sherertz RJ, Raad II, O'Grady N, Harris JS et al. Guidelines for the management of intravascular catheter-related infections. **Clin Infect Dis 2001**; 32:1249-1272.

67. Ruhe JJ, Menon A. Clinical significance of isolated *Staphylococcus aureus* central venous catheter tip cultures. **Clin Microbiol Infect 2006**; 12:933-936.

68. 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**; 191:2149-2152.

69. Moellering Jr RC. Vancomycin: a 50-year reassessment. **Clin Infect Dis 2006**; 42:S3-S4.

70. Cantú TG, Yamanaka-Yuen NA, Lietman PS. Serum vancomycin concentrations: reappraisal of their clinical value. **Clin Infect Dis 1994**; 18:533-543.

71. Elliot TS, Foweraker J, Gould FK, Perry JD, Sandoe JA. Guidelines for the antibiotic treatment of endocarditis in adults: report of the Working Party of the British Society for Antimicrobial Chemotherapy. **J Antimicrob Chemother 2004**; 54:971-981.

III

Endocarditis due to meticillin-resistant *Staphylococcus aureus* originating from pigs.

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Abstract

A 63-year-old woman with a kidney transplant was admitted with endocarditis caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Once her antibiotic therapy had been adjusted to the sensitivity pattern of the bacterial strain she recovered, without the need for surgical intervention. The isolated *S. aureus* was typed by multi-locus sequence typing as sequence type 398, a MRSA-strain that has been isolated from a high percentage of Dutch pigs. This is the first report of a life threatening infection with this pig MRSA. This strain is genetically different from the globally dispersed nosocomial MRSA-strains, and also from the strains that have been epidemic for several years in the USA as the causative agent of community-acquired skin infections. The Dutch Working Group on Infection Prevention (WIP) has adjusted its guidelines to halt spread of this strain within hospitals, and advises that the population at risk (pig breeders, slaughterhouse personnel and veterinarians) be held in isolation when hospitalised, until MRSA colonisation has been excluded. The patient described here, however, did not belong to this population at risk.

Humans are not the only reservoir for *Staphylococcus aureus*: numerous animals may serve as hosts.[1] In cows mastitis caused by *S. aureus* is the most prevalent infection,[2] and already in 1972 MRSA was found in milk from Belgian cows with mastitis. Through the years MRSA has been isolated from – amongst others – pigs, goats, dogs, cats, sheep, horses, rabbits, seals, chickens, turtles, bats and chinchillas. Until quite recently, however, MRSA-strains from animals closely resembled hospital-acquired MRSA (HA-MRSA) and transmission was believed to occur only from humans to animals, so-called "humanoses". Cases had been reported in which domestic pets had acquired MRSA from patients and had served as a reservoir for the bacterium, thus disrupting eradication therapy in their owners.

Primary MRSA-transmission from an animal source to humans was first detected in the Netherlands in July 2004. A 6-month-old girl was, unexpectedly, found MRSA-positive in her screening for *S. aureus* prior to elective thoracic surgery, although neither her nor her family had a history of admission to a foreign hospital (the main risk factor for MRSA in the Netherlands).[4] The MRSA-strain was not typable by standard pulsed field gel electrophoresis (PFGE) and could not be linked to any of the known outbreaks in the Netherlands. Multilocus sequence typing (MLST) classified it as sequence type (ST) 398.

A search for the source led to the girl's parents, pig farmers, who were also MRSA-positive, and ultimately to the pigs at the parents' farm: one of ten tested pigs was colonized with the same strain. Further research revealed that MRSA-colonization was quite common in Dutch pigs, pig-farmers and veterinarians;[4] 6 of 26 pig-farmers in the area were MRSA-carriers, and amongst 540 screened pigs in various Dutch slaughterhouses 39% was tested MRSA-positive.[5]

The pig-MRSA differed from hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) by its resistance to restriction endonuclease *SmaI* (which is applied for PFGE-typing of *S. aureus*), and by its particular susceptibility-pattern: it is susceptible to most antibiotics, but exhibits almost universal resistance to tetracyclines,[9,22] probably a result of the high amount of tetracyclines used as growth enhancers in pig-farming.

The first reports of human colonization by pig-MRSA was shortly followed by reports of human infections with the strain: in 2006 a case of mastitis and a case of an infected pig-bite wound were reported.[7,8] And also a case endocarditis, which is described below:

Case Report

A 63-year-old woman was admitted to the University Medical Center Utrecht in March 2006 with acute fever and confusion. According to her husband, she had developed confusion after a short period of nausea. No other complaints were mentioned.

The patient's medical history included chronic hypertensive kidney failure for which she received a kidney transplant in 1994, subclinical hyperthyroidism, a skin infection with an *Exophialia sp*., and avascular necrosis of the distal tibia. One month prior her renal function had deteriorated due to combined vascular and humoral rejection, she had been treated with anti-thymocyte globulines (ATG). ATG was through a temporary femoral intravenous catheter. When the catheter was removed, six days after insertion, purulence of the catheter exit site was noted, but neither the catheter tip nor the exit site were cultured.

Her medication at the time consisted of mycofenol acid 500 mg twice daily, prednisone 20 mg once daily, tacrolimus 2 mg twice daily, enalapril 20 mg once daily, atenolol 25 mg once daily and furosemide 80 mg once daily.

We saw an agitated, confused woman, with a blood pressure of 140/90 mmHg, a regular heartbeat of 90 beats/min, a body temperature of 38,7°C and a respiratory rate of 24/min. Further physical examination was unremarkable; in particular, no cardiac murmurs were noted. Laboratory tests showed a hemoglobin of 5,6 mmol/l; leukocytes of $14{,}9 \times 10^9$/l with immature forms; C-reactive protein (CRP) of 68 mg/l; creatinine of 181 µmol/l. After blood cultures were drawn, the preliminary diagnosis "sepsis of unknown origin in a immunocompromized patient" antibiotic therapy was started with intravenous ceftriaxone 2 g once daily.

After 24 hrs incubation, 3 of 4 blood cultures yielded *S. aureus*. At that time, since there was no other apparent focus of infection, the diagnosis of endocarditis was considered

and the antibiotic therapy was switched to flucloxacillin 2 g six times daily. Because of the renal insufficiency of the patient, aminoglycoside-therapy was withheld.

At first the instituted antibiotic therapy appeared to be successful: the inflammation-parameters dropped (Figure 1) and within 24 hours the patient's fever subsided, but on the sixth day of treatment she became feverish again. That same day the *S. aureus* isolated from her blood cultures proved methicillin-resistant. The first blood culture taken from the patient contained two populations; one with a minimal inhibitory concentration (MIC) for oxacillin of 2 µg/ml (susceptible) and a resistant subpopulation with an MIC of 8 µg/ml (resistant). From a blood culture taken after ten days of therapy with beta-lactam antibiotics a strain with an oxacillin MIC of 32 µg/ml was recovered, indication that induction of the strain's MecA-gene occurred in our patient. Possibly the initial susceptibility of the MRSA-strain is the explanation of the initial clinical response to therapy with ceftriaxone and flucloxacillin (Figure 1). The strain was resistant to ciprofloxacin and tetracycline, but susceptible to vancomycin, co-trimoxazole, rifampicin, fusidic acid, erythromycin and clindamycin. Flucloxacillin-therapy was therefore changed to vancomycin 1 gram / 48 hours intravenously and rifampicin 300 mg thrice daily.

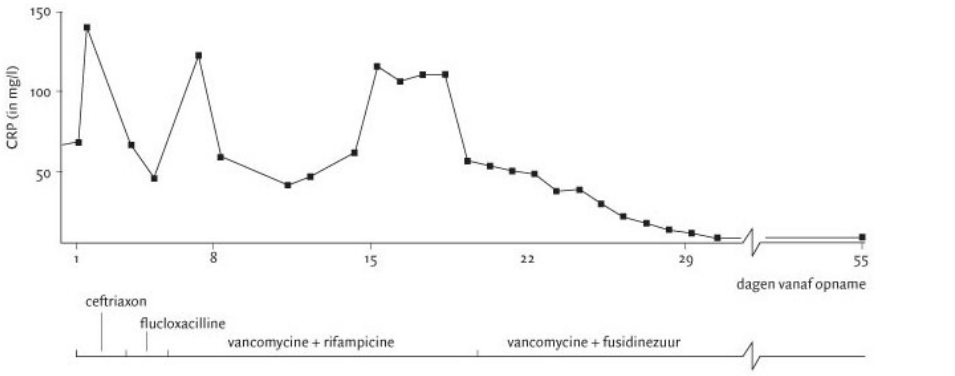

Figure 1: Antibiotic therapy and C-reactive protein concentration (CRP) in patient A from the day of hospitalization (day 1). On day 55 the antibiotic therapy was ended. On day 18 the interval of vancomycin administration was shortened from q48h to q36h.

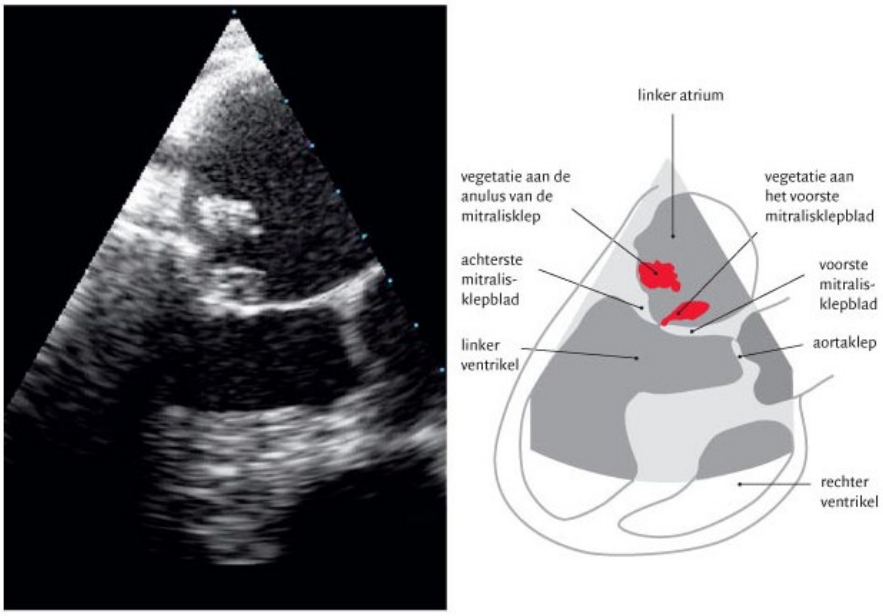

Figure 2: Transesophageal echocardiography of patient A on day 7 of hospitalization

Transesophageal echocardiography revealed a ring abscess of the mitral valve, a vegetation on this valve and a hole in the valve (Figure 2). It was decided not to operate the patient, because of the risks involved and because she remained hemodynamically stable.

A rise in the inflammation-parameters after seven days of treatment with vancomycin and rifampicin (day 13 since admission) prompted drawing of repeat blood cultures, which once again yielded MRSA, now resistant to rifampicin. The patients vancomycin trough level at that moment was 8 mg/l and probably inadequate for endocarditis-treatment: in 2006, trough levels of 10-15 mg were recommended for endocarditis[6] and more recent guidelines advise 15-20 mg/l.[23] The interval of vancomycin-administration was shortened to once per 36 hours and rifampicin was substituted by oral fusidic acid 120 mg twice daily. On the 17th day of admission for the first time a cardiac murmur (grade I/VI) was appreciated.

Under the instituted regimen the patient improved and her inflammation-parameters normalized; after 7 weeks the therapy was discontinued. A second echocardiography showed some rests of the vegetation and a moderate insufficiency of the mitral valve, but the patient did not suffer complications of MRSA-endocarditis during her follow-up period. Two years after this episode, however, she succumbed to causes unrelated to her episode of endocarditis.

Table 1: Toxin gene profile of 4 types of methicillin-resistant *Staphylococcus aureus*

	Multi locus sequence type			
	ST 398	**ST 45**	**ST 80**	**ST 8**
SCCmec gene	V	V	IV	IV
Toxin gene				
Panton-Valentine leukocidine	0	0	1	1
enterotoxin A	0	0	0	0
enterotoxin B	0	0	0	0
enterotoxin C3	0	1	0	1
enterotoxin E	1	1	1	1
enterotoxin G	0	1	0	0
enterotoxin I	0	1	0	0
enterotoxin J	0	1	1	0
α-hemolysin	1	1	1	1
β-hemolysin	1	1	1	1
γ-hemolysin	0	1	1	1
toxic shock syndrome toxin-1	0	0	0	1
leukocidine E	0	0	1	1
leukocidine M	0	1	0	0
bacteriocine A	0	1	1	1

SCCmec: staphylococcal chromosomal cassette with mecA gene; 0: gene absent; 1

Discussion

The lack of risk-factors for MRSA-carriage in our patient and the resistance pattern of the MRSA-strain (specifically resistance to tetracyclines), in combination with the fact that she resided in a rural area next to a pig-farm – she and her husband owned a chicken farm – made us suspect that the MRSA-strain might originate from pigs. Indeed the strain was not typable by PFGE, and belonged to MLST ST398. Furthermore, the strain distinguished itself by a divergent toxin-profile. Out of 15 toxin-genes present in HA-MRSA, only 3 were present in the pig-strain, while other HA-MRSA clones possess between 8 and 10 (Table 1). This might be indicative of a lower virulence of the pig-strain.

Figure 3 depicts an analysis of the international MRSA MLST-database in 2006. At that time, the database contained 816 different ST's, 490 with epidemiological data. As can be appreciated, ST 398 is not related to any of the five major clonal complexes (CC's) of MRSA, but forms a separate CC with 5 other ST's. The international MLST-collection included 2 other ST 398-isolates: 1 strain isolated in Groningen in 2003 and one isolated on the Republic of Cape Verde in 1997. Furthermore, there were 4 single-locus variants of ST 398 (ST's which differ in only 1of 7 genes of ST 398): 2 from The Netherlands, isolated in 2004 and 2005, and 2 from Germany, isolated in 1999 and 2001, the first of which was a methicillin-susceptible *S. aureus*. The database does not include details about these strains or the clinical circumstances in which they were isolated. Retrospectively, *S. aureus* ST 398 was isolated in France between 1996 and 2002 from 6 pig-farmers and 4 pigs; 1 of these isolates was MRSA.[12] These data indicate that spread of ST 398 and other closely related MRSA-strains occurred previous to 2004 and that spread was not limited to Dutch cattle.

Indeed, in the following years it became apparent that the pig-MRSA was not merely a Dutch epidemic; ST398 MRSA was found in Singapore,[24] Canada,[25] the United States[26] and throughout Europe. The strain had been widely and globally spread among pigs and calves for quite some time, with subsequent transmission of MRSA to caretakers, but its existence had only been noticed in the Dutch setting of a low "background MRSA-prevalence". As a result of the Dutch "search-and-destroy"-policy for MRSA, the prevalence of MRSA-colonization in the Dutch population is still under 0.1% (data from 2000).[16]

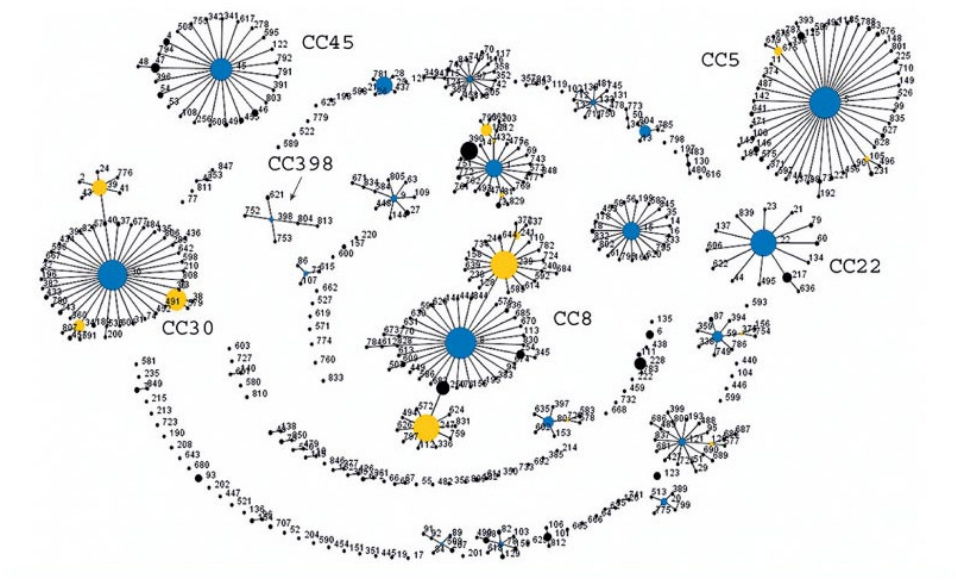

Figure 3: Relation between the different strains of methicillin-resistant *Staphylococcus aureus* (MRSA) in the international multilocus sequence type (MLST)-collection with 490 separate sequence types (www.mlst.net).

Every dot represents a sequence type. So-called "single locus variants" (SLV's) are connected by lines; the length of the line indicates the number of housekeeping genes that differ between two sequence types. The size of the dots indicates the number of strains with the identical MLST-type. The five pandemic clonal complexes (CC's) with the numbers 5, 8, 22, 30 and 45 are the most frequent. It can be appreciated that the MRSA-strain with sequence type 398, "the pig-strain" (indicated with an arrow), belongs to a small clonal complex with four SLV's (ST 621, 752, 753 and 804), and one double locus variant (ST813), and that it is not related to the five pandemic CC's.

The presented clinical case has regularly been cited as proof that pig-MRSA may cause severe infections, however, it could also be regarded as evidence that pig-MRSA is less virulent than hospital-acquired MRSA. Despite the fact that our patient was immunocompromized, and that she was initially treated with beta-lactam antibiotics for five days, and later several days with suboptimal dosages of vancomycin, she was cured, whereas mortalities of 29% to 100% have been reported for MRSA-endocarditis.[27,28] After the case above was published, other cases of invasive infections with ST398 MRSA followed – destructive otomastoiditis,[18] multiloculated abscesses,[19] ventilator-associated pneumonia[20] – but reports have remained scarce.

Still, the guidelines for prevention of MRSA-spread in Dutch hospitals by the Dutch Working Group on Infection Prevention (WIP) were adapted. Persons who have contact with live pigs or veal calves were classified as high-risk for MRSA-carriership (category 2); they now receive care in strict isolation until MRSA-screening cultures or MRSA-screening PCRs are negative.[21]

References

1. Lee JH. Methicillin (Oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. **Appl Environ Microbiol. 2003**;69:6489-6494.

2. Rainard P, Corrales JC, Barrio MB, Cochard T, Poutrel B. Leucotoxic activities of Staphylococcus aureus strains isolated from cows, ewes, and goats with mastitis: importance of LukM/LukF'-PV leukotoxin. **Clin Diagn Lab Immunol. 2003**;10:272-277.

3. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant Staphylococcus aureus USA 300 clone as the predominant cause of skin and soft-tissue infections. **Ann Intern Med. 2006**;144:309-317.

4. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in pig farming. **Emerg Infect Dis. 2005**;11:1965-6.

5. Vandenbroucke-Grauls CMJE, Beaujean DJMA. Meticillineresistente Staphylococcus aureus bij veehouders. **Ned Tijdschr Geneeskd. 2006**;150:1710-1712.

6. Horstkotte D, Follath F, Gutschik E, Lengyel M, Oto A, Pavie A, et al. Guidelines on prevention, diagnosis and treatment of infective endocarditis executive summary; the task force on infective endocarditis of the European society of cardiology. **Eur Heart J. 2004**;25:267-276.

7. Schneeberger P, Gend W van, Smiseth OA. Varken bijt boer. **Med Contact. 2006**;61:969.

8. Dijke B van, Koppen H, Wannet W, Huijsdens W, Neeling H, Voss A. Methicillin-resistant Staphylococcus aureus and pig-farming (abstract). **Clin Microbiol Infect. 2006**;12:S4.

9. Bens CC, Voss A, Klaassen CH. Presence of a novel DNA methylation enzyme in methicillin-resistant Staphylococcus aureus isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. **J Clin Microbiol. 2006**;44:1875-1876.

10. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. **Antimicrob Agents Chemother. 2003**;47:3926-3934.

11. 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 USA. 2002**;99:7687-7692.

12. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. **Emerg Infect Dis. 2005**;11:711-714.

13. Melles DC, Hendriks WDH, Ott A, Verbrugh HA. Uitbraak van meticillineresistente Staphylococcus aureus (MRSA) in de regio Rijnmond: grootste MRSA-epidemie in Nederland. **Ned Tijdschr Geneeskd. 2004**;148:1038-1043.

14. Flier M van der, Dijk NB van, Fluit AC, Fleer A, Wolfs TFW, Gestel JPJ van. Fatale pneumonie bij een adolescent door thuis opgelopen meticillineresistente Staphylococcus aureus positief voor Panton-Valentine-leukocidine. **Ned Tijdschr Geneeskd. 2003**;147:1076-1079.

15. Berger-Bachi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. **Arch Microbiol. 2002**;178:165-171.

16. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CMJE, Meester MH, et al. Low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. **J Hosp Infect. 2004**;56:321-325.

17. Stelfox HT, Bates DW, Redelmeier DA. Safety of patients isolated for infection control. **JAMA. 2003**;290:1899-1905.

18. Van Hoecke H, Piette A, De Leenheer E, Lagasse N, Struelens M, Verschraegen G, Dhooge I. Destructive otomastoiditis by MRSA from porcine origin. **Laryngoscope. 2009**;119:137-140.

19. Pan A, Battisti A, Zoncada A, Bernieri F, Boldini M, Franco A, et al. Community-acquired methicillin-resistant Staphylococcus aureus ST398 infection, Italy. **Emerg Infect Dis. 2009**;15:845-847.

20. Witte W, Strommenger B, Stanek C, Cuny C. Methicillin-resistant Staphylococcus aureus ST398 in humans and animals, Central Europe. **Emerg Infect Dis. 2007**;13:255-258.

21. Werkgroep Infectiepreventie. MRSA, ziekenhuis. **www.wip.nl**: March 2008

22. Wulf MW, Sørum M, van Nes A, Skov R, Melchers WJ, Klaassen CH, Voss A. Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. **Clin Microbiol Infect. 2008**;14:29-34.

23. Rybak MJ, Lomaestro BM, Rotschafer JC, Moellering RC, Craig WA, Billeter M, et al. Vancomycin therapeutic guidelines: a summary of consensus recommendations from the infectious diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. **Clin Infect Dis. 2009**;49:325-327

24. Sergio DM, Koh TH, Hsu LY, Ogden BE, Goh AL, Chow PK. Investigation of meticillin-resistant Staphylococcus aureus in pigs used for research. **J Med Microbiol. 2007**;56:1107-1109.

25. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. **Vet Microbiol. 2008**;128:298-303.

26. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, et al. Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. **PLoS One. 2009**;4:e4258.

27. Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. **JAMA. 2005**; 293:3012-21.

28. Grothe C, Belasco A, Bettencourt A, Diccini S, Vianna L, Pignatari A, et al. Lethality of endocarditis due to *Staphylococcus aureus* among patients on hemodialysis. **Nephrol Nurs J. 2009**; 36:613-619.

IV

Quantifying the relationship between *Staphylococcus aureus* bacteremia and *Staphylococcus aureus* bacteriuria.

A retrospective analysis in a tertiary care hospital.

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Abstract

In this retrospective cohort study, patients who had *Staphylococcus aureus* bacteremia but who lacked signs and symptoms of urinary tract infection due to *S. aureus* and who did not have an indwelling urinary catheter had a likelihood of *S. aureus* bacteriuria of 2.5% (2 of 79 patients). Therefore, we strongly question the theory that *S. aureus* bacteremia causes *S. aureus bacteriuria*.

Introduction

It is popularly believed that *Staphylococcus aureus* bacteremia causes *S. aureus* bacteriuria, especially in the case of endocarditis.[1-3] On the basis of this assumption, it is often advised to seek a distant site of infection as a source of (transient) bacteremia in the case of *S. aureus* bacteriuria. However, data that confirm this widely assumed association are scarce, and among 39 patients with *S. aureus* bacteriuria (but without demonstrated bacteremia), a distant site of *S. aureus* infection was demonstrated in only 1 patient.[4] Moreover, in the most frequently cited study in this regard,[5] an association between *S. aureus* bacteremia and *S. aureus* bacteriuria was reported, but causality was not claimed. In fact, it was concluded that "staphylococcal bacteriuria appears to be a frequent and unexplained concomitant of *S. aureus* bacteremia" (p. 306).

If the theory that *S. aureus* bacteriuria is a reflection of bacteremia is not true, adherence to it may lead to extensive and unnecessary "source investigations" in patients with *S. aureus* bacteriuria and to disregarding the urinary tract as a focus of *S. aureus* bacteremia. *S. aureus* is, however, known to cause urinary tract infection (UTI), which may well be the source of *S. aureus* sepsis syndrome.[6] Furthermore, it has been demonstrated that urinary catheters predispose to urinary colonization with *S. aureus*[6-8] and that colonization with this microorganism is a risk factor for subsequent infection.[6,9]

In this study, we aimed to quantify the relationship between *S. aureus* bacteremia and *S. aureus* bacteriuria. We hypothesized that, if *S. aureus* bacteremia truly causes *S. aureus* bacteriuria, we should observe this phenomenon in patients without UTI and without risk factors for urinary colonization with *S. aureus* (specifically, the presence of urinary catheters), because only such could, in our opinion, be unambiguously attributed to *S. aureus* bacteremia.

Methods

Patients who received a diagnosis of documented *S. aureus* bacteremia during the period from 1 June 2001 to 1 June 2006 were identified in the database of the bacteriology department of the University Medical Center Utrecht (a tertiary care hospital in Utrecht, The Netherlands, with 1024 beds). From these patients, 2 groups were selected: patients who had a urine sample obtained for culture on the same day as a positive result of blood culture result (group 1), and patients who had *S. aureus* bacteriuria on any other day during this 5-year period (group 2), with an isolate with a similar susceptibility pattern (difference in susceptibility of ≤1 antibiotic between the urine and blood isolates). Patients with a clinical diagnosis of *S. aureus* UTI were excluded from groups 1 and 2 and analyzed separately, because in these cases, the bacteriuria was regarded as the cause of *S. aureus* bacteremia rather than its effect.

For each patient, we recorded the age, the number of positive results of blood culture bottles in a window period of 2 days before until 2 days after obtaining urine for culture, results of urine cultures, and presence of an intravesical catheter. The definite source of *S. aureus* bacteremia was established on the basis of patient charts. In patients with a clinical diagnosis of endocarditis, we evaluated whether the case complied with the Duke criteria (10).

The diagnosis "obvious UTI" was defined by the following criteria (all 5 criteria had to be met): (I) UTI diagnosed by the clinician, (II) presence of leukocyturia, (III) no other probable focus of bacteremia, (IV) presence of *S. aureus* bacteriuria (difference in susceptibility of ≤1 antibiotic between urine and blood isolate), and (V) presence of either localizing symptoms, a recent surgical procedure or manipulation in the urinary tract, or urinary obstruction. The medical charts of these patients were examined for previous invasive urologic procedures and for urologic neoplasms.

Blood cultures were incubated for a minimum of 5 days at 35°C in an automated detection system. Urine specimens were processed with a dip slide (Uricult; Orion Diagnostics), and, when the results were positive ($\geq 10^4$ cfu/mL), they were subcultured onto blood agar. Identification and susceptibility testing were performed using an automated system and software; the identification was confirmed by testing free and bound coagulase.

The associations between *S. aureus* bacteriuria and the presumed risk factors were tested for statistical significance by χ^2 analysis and χ^2 analysis for trend, using SPSS software, version 12.0 (SPSS).

Table 1: Clinically identified focus of bacteremia in Group 1 and group 2.

Focus of bacteremia	Group 1 (n=153)		Group 2 (n=23)
	Urine culture neg (n=141)	Urine culture pos (n=12)	
Endocarditis	11 (8%)	2 (17%)	1 (4%)
Intravascular line infect.	39 (28)	3 (25)	5 (22)
Phlebitis /Infected endovascular prothesis	19 (13)	-	2 (9)
Skin / Wound inf	9 (6)	-	-
Abscess	12 (9)	-	-
Other	21 (15)	3 (25)[a]	4 (17)
Unknown	30 (21)	4 (33)[b]	11 (48)[c]

[a]the diagnoses in these three patients were: osteomyelitis, spondylodiscitis and upper respiratory tract infection; [b]of the 4 patients with no clinically identified focus for the *S. aureus* bacteremia, 1 complied with criteria II-V for UTI; [c]of the 11 patients with no clinically identified focus for the *S. aureus* bacteremia, 6 complied with criteria II-V for UTI.

Results

In the studied 5-year period, a total of 64,256 urine samples were processed at our laboratory. Of the 16,667 samples with positive urine culture results, 8928 were midstream urine samples, and 7739 were catheter-obtained samples. Six hundred eighty-five urine cultures (4.1% of all positive culture results) yielded *S. aureus*; 222 were midstream urine cultures (2.5% of all midstream urine cultures with positive results), and 463 were catheter cultures (6.0% of all catheter cultures with positive results); samples were obtained from 470 patients (167 patients for midstream urine samples and 303 patients for catheter-obtained samples). During this period, 42,101 sets of blood samples for culture were obtained, of which 5519 yielded positive results, and 950 yielded *S. aureus*, from 515 patients. In the total group of patients with *S. aureus* bacteriuria (n=470), 64% had urinary catheters in place, and 9.6% had *S. aureus* bacteremia. This confirms previous observations that intravesical catheters are an important risk factor for *S. aureus* bacteriuria (Table 1).

In group 1 (Table 2), 153 patients had urine culture samples obtained on the same day that *S. aureus* bacteremia was detected and did not have a diagnosis of UTI; of these culture samples, 79 were midstream urine samples, and 74 were catheter-obtained urine samples. Twelve patients (7.8%) had *S. aureus* bacteriuria; 10 of these patients had intravesical catheters. The likelihood of *S. aureus* bacteriuria in the case of *S. aureus* bacteremia and in the absence of a catheter was, therefore, 2.5% (2 of 79 patients; Figure 1). Of note, in 1 of these 2 patients (a patient undergoing hemodialysis who had oliguria and renal failure associated with glomerulonephritis), it was questionable whether *S. aureus* bacteriuria could be attributed to *S. aureus* bacteremia. The only independent risk factor for *S. aureus* bacteriuria among patients with *S. aureus* bacteremia was the presence of indwelling urinary catheters (OR, 6.0; 95% CI, 1.1–41.4). We found no association of *S. aureus* bacteriuria with age, endocarditis, or number of positive blood culture results.

In group 2 (table 2), 23 patients had *S. aureus* bacteremia and *S. aureus* bacteriuria on different days. Eighteen patients (78%) had urinary catheters in place. In 13 patients, documented *S. aureus* bacteriuria preceded *S. aureus* bacteremia (median interval, 5 days;

range, 1–140 days), and in 10 patients, *S. aureus* bacteremia preceded *S. aureus* bacteriuria (median interval, 19 days; range, 1–162 days; Figure 2).

Five patients in group 2 did not have urinary catheters in place. Of these patients, 1 had severe kidney disease (acute rejection of a kidney transplant) and was oliguric; in 2 patients, the urine culture results became positive >3 months after *S. aureus* bacteremia; and in 1 patient, bacteriuria (which occurred 5 days before *S. aureus* bacteremia and sepsis syndrome) may have indicated UTI. This leaves only 1 patient without an identified focus for *S. aureus* bacteremia in which "unexplained" *S. aureus* bacteriuria might have been attributable to the bacteremia.

Sixteen (31%) of the 51 patients with *S. aureus* bacteremia and *S. aureus* bacteriuria fulfilled the criteria for diagnosis of *S. aureus* UTI. Twelve of these patients (75%) had urinary catheters in place, 12 (75%) had previously undergone invasive urologic procedures (such as changing of a nephrostomy catheter, placement of a suprapubic urine catheter, radical cystectomy, transurethral resection of the prostate, and renal transplantation), and 8 (50%) had been given a diagnosis of urologic neoplasms.

Seven of the 46 patients with *S. aureus* bacteremia with an "unknown origin" fulfilled criteria 2–5 for UTI but lacked the treating physician's diagnosis to be qualified as such. Pyuria and bacteriuria were demonstrated in the week previous to the bacteremia in 6 patients and on the same day in 1. Six of these patients had urinary catheters in place.

Table 2: Relation between *S. aureus* bacteriuria, SAB and UTI in different studies

Study (number of patients with *S. aureus* bacteriuria)	Percentage of bacteriuric patients who had a catheter	Percentage of bacteriuric patients who suffered SAB
Demuth e.a. 1979 (n=127)	73%[a]	15%
Arpi e.a. 1984 (n = 132)	63%	9.0%
Muder e.a. 2006 (n = 102)	82%	13-21%[b]
This study (n = 470)	64%	9.6%

[a]this group was defined as "recent urinary catheterization or invasive procedure"; [b]21% is including late occuring SAB (>1 week after original urine culture)

Figure 1: Flow chart depicting the relation between *Staphylococcus aureus* bacteremia and *S. aureus* bacteriuria

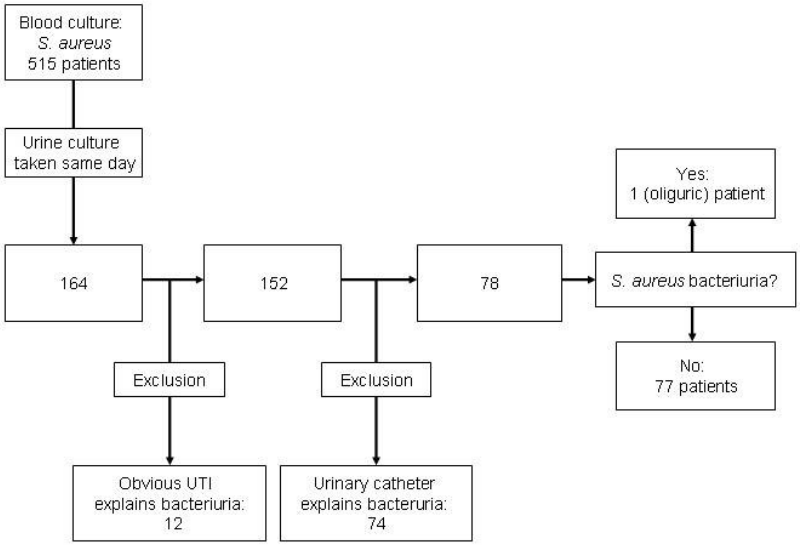
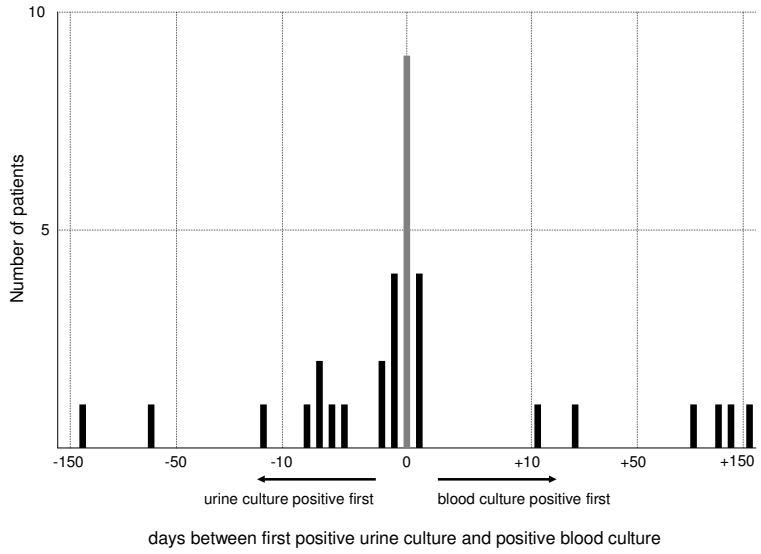


Figure 2: Temporal relation between demonstrated *Staphylococcus aureus* bacteremia and *S. aureus* bacteriuria



Discussion

Our study is, to our knowledge, the first that specifically tried to identify patients with *S. aureus* bacteremia and *S. aureus* bacteriuria who did not have obvious risk factors for *S. aureus* bacteriuria (specifically, no UTI and no indwelling catheter in place). Of the 79 patients with *S. aureus* bacteremia who lacked these 2 risk factors, only 2 had positive urine culture results on the day that blood samples were obtained for culture; 1 additional patient had *S. aureus* bacteremia and *S. aureus* bacteriuria identified in a midstream urine specimen on separate days. We identified 7 patients who fulfilled all of the criteria for UTI except for criterion 1 (i.e., diagnosis made by the treating clinician). One may speculate that this finding suggests that *S. aureus* UTI was underdiagnosed as a cause of *S. aureus* bacteremia.

Because of its retrospective nature, this study has several limitations. Urine samples were not obtained in a protocolized manner, and we could analyze the diagnostic samples only as ordered by the treating physicians; this probably biased our observations toward a higher percentage of patients with UTI. Furthermore, it cannot be excluded that blood samples were obtained for culture first and that urine samples were obtained after antibiotic therapy had been started, therefore yielding false-negative results. Yet all of the previous studies also had this limitation—and probably to a larger extent, because wider intervals (up to 48 h) were accepted. Finally, only the diagnoses of UTI and endocarditis were confirmed on the basis of predetermined criteria.

In short, on the basis of the results of this study—and the evidence in literature—we refute the widely held belief that *S. aureus* bacteriuria is a common effect of *S. aureus* bacteremia and that *S. aureus* bacteriuria in itself justifies extensive source investigations. A prospective study is, however, required to provide a definitive answer to this question.

References

1. Archer GL. Staphylococcal infections. In: Goldman L, Ausiello D, eds. Cecil textbook of medicine. 22nd ed. Philadelphia: WB Saunders, **2003**:1803–9.

2. Fowler VG Jr, Sexton DJ. Complications of *Staphylococcus aureus* bacteremia. **UpToDate online 2006**; ed. 14.2. www.utdol.com. Accessed 4 December.

3. Lowry FD. Staphylococcal infections. In: Kasper DL, Braunwald E, Fauci AS, et al, eds. **Harrison's principles of internal medicine 2005**; 16th ed.:814–23.

4. Sheth S, DiNubile MJ. Clinical significance of *Staphylococcus aureus* bacteriuria without concurrent bacteremia. **Clin Infect Dis 1997**; 24:1268–9.

5. Lee BK, Crossley K, Gerding DN. The association between *Staphylococcus aureus* bacteremia and bacteriuria. **Am J Med 1978**; 65:303–6.

6. 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**; 42:46–50.

7. Arpi M, Renneberg J. The clinical significance of *Staphylococcus aureus* bacteriuria. **J Urol 1984**; 132:697–700.

8. Demuth PJ, Gerding DN, Crossley K. *Staphylococcus aureus* bacteriuria. **Arch Intern Med 1979**; 139:78–80.

9. 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**; 23:495–501.

10. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. **Clin Infect Dis 2000**; 30:633–8.

V

The Relationship between Bacterial Colonization of External Cerebrospinal Fluid Drains and Secondary Meningitis

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Abstract

A retrospective study over an 8 year period was conducted at the UMC Utrecht to assess the predictive value of a positive culture from a CSF-drain tip for the development of secondary meningitis. A total of 139 patients with positive cultures of CSF-drains were included; 72 patients (52%) suffered secondary meningitis at the time of CSF-drain removal, or developed it consecutively. Development of secondary meningitis was associated with use of ventricular drains, with age <18 years and with colonization with *Staphylococcus aureus*. Thirty-two patients were diagnosed with secondary meningitis 24 hrs or more after CSF-drain removal; in thirteen patients (18%) the diagnose was made after 48 hrs or more. A positive CSF-drain culture therefore appears strongly associated with the development of secondary meningitis.

Introduction

External ventricular drains (EVDs) and external lumbar drains (ELDs) are commonly employed in neurosurgery to control hydrocephalus, to monitor intracranial pressure, and to correct cerebrospinal fluid (CSF) leakage of surgical wounds. They are usually placed in one of the cerebral ventricles or in the lumbar subarachnoid space but may also be placed in an intracranial cyst. A major complication of CSF-drains is secondary meningitis and ventriculitis, a condition associated with high morbidity and sometimes even mortality.[1] Secondary meningitis is most commonly caused by coagulase-negative staphylococci (CoNS) (50%-90% of all cases), and by *Staphylococcus aureus* (13%-27%). Other organisms implicated include Gram-negative rods, and Gram-positive rods such as *Proprionibacterium acnes* and *Corynebacterium spp*.[2,8] Risk factors for the development of secondary meningitis include intracranial hemorrhage, and cranial fractures.[9,10] Previous neurosurgical operations, ventriculostomy irrigations, systemic infection, and duration of catheterization have also been implicated as risk factors.[6,9,10]

The incidence of these drain-related infections has been reported between 2 and 22% (mean 8,8%) in patients with EVDs,[7] and between 4 and 7% in patients with ELDs. The large variation in incidence may reflect differences in drain handling procedures[5] and patient-population, but is also partly due to the lack of a uniform definition for drain-associated meningitis. The criterium of a single positive CSF-culture is the most widely employed; however, some studies incorporate pleiocytosis of the CSF, low CSF glucose or neurological symptoms,[3] as does the definition by the Centers of Disease Control and Prevention (CDC).[4]

Different routes of CSF-drain contamination and infection are possible: contamination of the drain during insertion, contamination of the drain system during manipulations, colonization of the drain at the insertion site by skin flora, and neurosurgical site infection (which may lead to contamination of the drain directly through the CSF or through the infected soft tissue).[5] In most of these routes of infection it may be expected that

CSF-drain colonization will precede drain-associated meningitis, and that culture of the CSF-drain tips will yield the bacterial pathogen responsible for the drain-associated infection.

At our laboratory we regularly receive the tips of CSF-drains for bacteriological culture, although the diagnostic and predictive value of these cultures has thus far not been established. The aim of this study was to determine the predictive value of CSF-drain colonization for the development of drain-associated meningitis and to determine associated risk factors.

Patients and Methods

Patients and patient data

The study was conducted at the University Medical Center Utrecht, a 1042-bed teaching hospital. Patients with colonized CSF-drains during the period June 2001 – June 2009 were identified in the database of the microbiology department. From the same database, culture results of CSF-drains and cerebrospinal fluid were collected. Patient charts were reviewed to retrieve demographical, clinical and laboratory data. The following data were extracted from the patient charts: age, sex, underlying neurological condition and indication for drain implantation, type of CSF-drain, dates of drain insertion and extraction, risk factors for infectious disease (malignancy, hematological malignancy, immunosuppressive therapy, autoimmune disease, diabetes mellitus, and mechanical ventilation), systemic and local signs of inflammation, neurological symptoms indicative of secondary meningitis, antibiotic usage around the time of CSF-drain removal, 3-month survival and – if applicable – cause of death. To estimate the percentage of CSF-drains cultured, all CSF-drain insertions in the operating room (i.e. all EVDs and a part of the ELDs) were retrieved from the hospital database, and compared with the database of the microbiology department to calculate the percentage of drain tips which had been cultured. Patients in which the diagnosis secondary meningitis had been made before placement of the CSF-drain were excluded from the study.

Definitions

The clinical diagnosis of secondary meningitis was defined as (1) documentation of the diagnosis in the patient charts and (2) start of antibiotic therapy directed at secondary meningitis. In our hospital standard empiric therapy consisted of either flucloxacillin 2 grams every 4 hours in combination with ceftriaxone 2 grams every 12 hours, or vancomycin 1 gram every 12 hours in combination with ceftazidim 2 grams every 8 hours. The day antibiotic therapy was initiated was considered the day of diagnosis. The presence of neurological symptoms was defined as documentation of one or more of the following: (1) nuchal rigidity, (2) decrease in Glasgow Coma Scale (GCS), (3) headache, or (4) nausea with vomiting.

Culture

Prior to drain removal, the skin around the entry site was desinfected with 70% ethanol or with an ethanol-based desinfection gel. The drains tips were deposited in sterile containers for transportation to the microbiology laboratory, where they were processed by rolling them several times over trytic soy agar with sheep blood (Oxoid) and GC-lect agar (BD).The agar plates were incubated for 48 hours at 35º C, and were read a first time after overnight incubation. For *S. aureus* and Gram-negative bacteria any number of colony-forming units was considered positive; for CoNS and Gram-positive bacteria other than staphylococci an arbitrary cut-off of ≥15 colony-forming units was applied. To minimize manipulations of the drain-systems and prevent the development of secondary meningitis, the protocol for culture of CSF at our institution is to culture only when the CSF-drain is removed or when secondary meningitis is suspected.

Statistical analysis

Data was analyzed using SPSS for Windows (version 15.0). Nominal variables were analyzed by chi-square test and Fisher's exact test where appropriate, continuous variables were analyzed by the Mann-Whitney *U* test. Significance was assessed two-sided for all variables, applying a cut-off value of $p<0.05$. Statistically significant risk factors in the univariate analysis were subsequently analyzed by multivariate logistic regression with both forward and backward stepwise analysis.

In patients with mixed cultures of their CSF-drain tip, the "most virulent" micro-organism was used for analysis. *S. aureus* was considered the most virulent, followed by Gram-negative bacteria, coagulase-negative staphylococci (CoNS) and finally other Gram-positive bacteria.

Results

During the 8-year study period, 839 EVDs and 202 ELDs were placed in the operating room. Of these, 393 (38%) were cultured and 83 were positive (21% of all cultured CSF-drains: 70 EVDs, 13 ELDs). From the microbiology database an additional 62 culture-positive ELDs were identified which had been placed on the neurology or neurosurgery wards. Six patients in which the diagnosis secondary meningitis was made before placement of the later culture-positive drain were excluded (3 EVDs and 3 ELDs), leaving a total of 139 patients with colonized CSF-drains (67 EVDs and 72 ELDs) for analysis.

The median age of the patients was 51 years (range 38 days - 80 years); 16 patients (12%) were younger than 18 years. 71 (51%) of the patients were male. The median time CSF drains remained in situ was 11 days. CoNS were cultured in 68 (49%) of the cases, *S. aureus* in 23 (16%), Gram-negative bacteria in 17 (12%) and Gram-positive bacteria other than staphylococci in 30 (22%). One drain yielded *Candida albicans*. Twenty-two cultures (16%) yielded more than one micro-organism.

Seventy-two patients (52%) were clinically diagnosed with secondary meningitis. Sixty-nine of these cases (96%) complied with the CDC-criteria for healthcare-associated meningitis.[4] In 58 (81%) of these patients a pathogen was cultured from the CSF (Table 1). CSF culture was negative in 10 patients with a clinical diagnosis of secondary meningitis; five of these patients received antibiotic therapy at the time CSF was drawn. In four other patients CSF was not cultured.

Three patients were diagnosed with secondary meningitis, but did not meet the CDC-criteria for meningitis: one patient with fever and a decrease in GCS died the day after removal of the CSF drain. No CSF samples were taken before death. Two other patients experienced fever and nuchal rigidity, but did not have CSF samples taken for culture or chemistry. In three patients who did not have neurological symptoms, positive cultures of spinal fluid were regarded as contamination and antibiotic treatment was not instituted. The first patient was discharged before the CSF results were known and was subsequently lost to follow up. The second patient was transferred to another hospital on the day of drain removal. The results of the CSF samples taken on the same day were not yet known at that point. For both these patients the CSF culture yielded a CoNS. The third patient was discharged five days after drain removal. The CSF culture yielded a *Corynebacterium sp.* for which the patient did not receive antibiotic treatment.

Seventeen of the 23 patients (74%) from whose CSF-drain tips a *S. aureus* was cultured were diagnosed with secondary meningitis. This was the case in 65%, 48% and 37% of colonization with Gram-negative bacteria, CoNS and other Gram-positive organisms respectively. In 55/58 patients (95%) with secondary meningitis and a positive CSF culture the micro-organisms cultured in the CSF and from the drain tip were identical in 55 patients. In 3 patients (5%) they differed: in one patient with CoNS cultured from the drain tip a *Citrobacter species* was cultured from the CSF; in two other patients the CSF culture yielded CoNS while the drain tips were positive for *Enterobacter sp.* and *Corynebacterium sp.* respectively.

In the univariate analysis, prognostic factors associated with secondary meningitis were: *S. aureus* cultured from the tip, EVD, intracranial hemorrhage, CSF-leakage and duration of catheterization (Table 2). In subsequent multivariate logistic regression only placement of an EVD, colonization with *S. aureus* and age under 18 remained independent risk factors. Intracranial hemorrhage, CSF-leakage and duration of catherization lost significance when correcting for presence of an EVD.

Twenty-seven patients (37%) were diagnosed with secondary meningitis prior to the drain removal, 13 (18%) patients on the day of shunt removal, 19 (26%) the day after

Table 1 (below): Signs and Symptoms of clinically diagnosed secondary meningitis

Variable	Secondary meningitis (n=72)	No secondary meningitis (n=67)
Documented neurological symptoms	43 (60%)	4 (6%)
Nuchal rigidity	22 (31%)	0 (0%)
Decrease in GCS	18 (25%)	2 (3%)
Headache or Vomiting	11 (15%)	2 (3%)
WBC count, mean	14.1	11.9
Fever > 38º C	37 (51%)	12 (18%)
SIRS	37 (51%)	10 (15%)
Positive CSF culture	58 (81%)	3 (4%)
Positive CSF chemistry		
1. Pleocytosis > 10 leucocytes/µl	61 (85%)	21 (31%)
2. Pleocytosis WBC:RBC ratio > *1:100*	57 (79%)	18 (27%)
3. Glucose < 2.4mmol/L	22 (31%)	3 (4%)
4. Protein > 0.4g/L	58 (81%)	25 (37%)
Combined 1, and either 3 or 4	56 (78%)	20 (30%)
CDC-criteria for meningitis [4]	69 (96%)	3 (4%)

Notes: GSC: Glasgow Coma Scale; WBC: White blood cell count; RBC: Red blood cell count; CSF: cerobrospinal fluid; SIRS: Systemic inflammatory response syndrome (≥2 of the following: temperature >38ºC or <36ºC; WBC count > 12000 cells/mm^3 or with >10% immature forms; heart rate >90 beats per minute; respiratory rate >20 breaths per minute); CDC: Centers for Disease Control and Prevention .

Table 2 (opposite page): Risk factors for secondary meningitis in patients with a colonized CSF-drain tip. *Notes***:** *EVD: external ventricular drain; ELD: external lumbar drain; ICP management: intracranial pressure management (either drainage or monitoring). IQR: interquartile range; Neurological symptoms: one of the following: nuchal rigidity, decrease in Glasgow Coma Scale, headache or vomiting. CoNS: coagulas-negative staphylococci *Odds ratio of EVD vs. ELD. **There was overlap between the different indications.*

Variable	Secondary meningitis (n=72)	No secondary meningitis (n=67)	Odds ratio (95%CI)	p-value (univar. analysis)	p-value (multivar. analysis)
Age, median years (IQR)	47.5 (24-59)	52.4 (44-65)			
Age < 18 years	13 (18%)	3 (5%)	4.7 (1.3-17.3)	0.012	0.015
Male sex	41 (57%)	30 (45%)		*n.s.*	
Co morbid conditions					
Underlying malignancy	19 (26%)	25 (37%)		*n.s.*	
Hematological malignancy	1 (1%)	2 (3%)		*n.s.*	
Diabetes mellitus	5 (7%)	5 (7%)		*n.s.*	
Immunosuppressive therapy	23 (32%)	15 (22%)		*n.s.*	
Autoimmune disease	1 (1%)	5 (7%)		*n.s.*	
Mechanical ventilation	8 (11%)	10 (15%)		*n.s.*	
Type of drain EVD	45 (62%)	22 (33%)	3.4 (1.7-6.8)*	<0.001	<0.001
ELD	27 (38%)	45 (67%)			
Duration of catheterization (mean)	11.3 days	10.7 days		0.017	*n.s.*
Indication for CSF-drainage**					
CSF leakage	14 (19%)	25 (37%)	0.4 (0.2-0.9)	0.019	*n.s.*
Intracranial hemorrhage	39 (54%)	23 (34%)	2.3 (1.2-4.6)	0.015	*n.s.*
Tumor	18 (25%)	27 (40%)		*n.s.*	
Traumatic brain injury	5 (7%)	6 (9%)		*n.s.*	
Abscess	1 (1%)	1 (1%)		*n.s.*	
Craniotomy	35 (49%)	37 (55%)		*n.s.*	
Pathogen					
Staphylococcus aureus	17 (24%)	6 (9%)	3.1 (1.2-8.5)	0.020	0.010
Gram-negative bacteria	11 (15%)	6 (9%)		*n.s.*	
CoNS	33 (46%)	35 (52%)		*n.s.*	
Other Gram-positive	11 (15%)	19 (28%)		*n.s.*	
Multiple micro-organisms	13 (18%)	9 (13%)		*n.s.*	

shunt removal and 13 (18%) patients were diagnosed with secondary meningitis two or more days (range 2-16 days) after CSF-drain removal (figure 1). Nine patients who developed a secondary infection two days or more after drain removal had CoNS colonization of their CSF-drain (69%), the other four concerned *Staphylococcus aureus*, *Enterobacter cloacae, Streptococcus sp.* and *Bacillus cereus*. In one patient the diagnosis was made 7 days after the colonized EVD was replaced by a ventriculo-peritoneal drain. A CSF-sample, drawn two days after the drain replacement, yielded CoNS. At first this bacterial growth was discarded as probable contamination; however, the diagnosis secondary meningitis was made over the course of the following days based on the patient's symptoms and a rise in CSF leucocyte count. In another patient the diagnosis secondary meningitis was made 16 days after removal of the colonized EVD (no new neurological drains were placed). The patient had intermittent periods of fever without an evident focus and developed nuchal rigidity after 16 days with CSF pleocytosis, reduced CSF glucose and increased CSF protein.

All cause 3-month mortality was 13% (n = 9) in patients who developed secondary meningitis and 3% (n = 2) in patients who did not develop secondary meningitis. Most patients succumbed to their underlying illness after completion of their antibiotic treatment and resolution of the signs and symptoms of secondary meningitis. In two patients however, death may have been partly attributable to the drain-associated meningitis. The first patient was admitted with intraventricular hemorrhage and suffered recurrent meningitis caused by a *Citrobacter sp.*; this patient's treatment was discontinued because of his poor neurological prognosis. A second patient (one of the three patients with clinically diagnosed meningitis who did not fullfill the CDC-criteria) suffered from an intracerebral non-Hodgkin lymphoma, possibly complicated by secondary meningitis; also this patient's treatment was stopped in view of a deteriorating clinical condition and a poor prognosis.

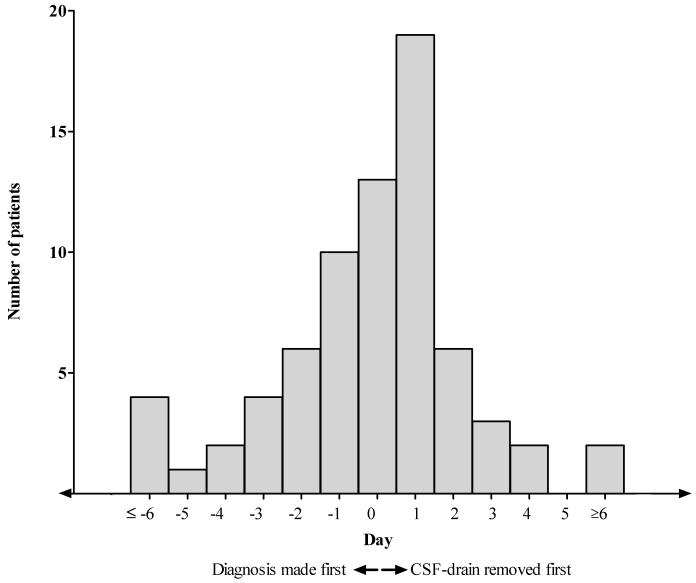

Figure 1: Day of diagnosis of secondary meningitis compared to the day of cerebrospinal fluid (CSF) drain removal in patients with bacterial colonization of the CSF-drain. Day 0 represents the day the CSF-drain was removed. Most diagnoses were made on day 1, i.e. one day after removal of the drain.

Discussion

In this study, 52% of the patients with demonstrated colonization of the CSF-draintip, were diagnosed with secondary meningitis. Within this group, development of secondary meningitis was significantly associated with *S. aureus* colonization, age below 18 years and EVD-placement.

In most cases the diagnosis secondary meningitis was made before the results of the CSF-drain culture became apparent, which is typically the day after removal. However, in 32 / 72 patients (44%) the diagnosis was made 24 hrs or more after removal of the CSF-drain and in 13 patients (18%) 48 hrs or more after removal. Therefore, positive culture results of CSF-drains should heighten alert for secondary meningitis. Various explanations for the development of meningitis after drain-removal may be considered. First of all, it is possible that in some cases signs of secondary meningitis are so mild that they are neglected by the clinician until a drain-culture or cerebrospinal fluid culture (often drawn the day of removal) is positive. Secondly it is possible that a low-grade drain-associated infection worsens due to discontinuation of the drainage when a CSF-drain is removed. Furthermore, a drain may be removed in view of a progressing infection which only gradually becomes apparent. Lastly, it could even be possible that removal of external CSF-drains causes secondary meningitis due to scraping of the micro-organism biofilm into the CSF-space, a mechanism which may also be applicable in particular cases of central intravenous catheter removal.[11]

Due to its retrospective nature this study suffers from several limitations. Whether or not a CSF-drain was cultured was decided by the clinician, and only 38% of CSF-drains were cultured. It is likely that the CSF-drains of patients which were more prone to development of secondary meningitis and patients who were already suspected of having secondary meningitis were more often cultured. Furthermore, a positive culture may have been interpreted by the clinicians as evidence that a patient suffered secondary meningitis. These limitations may have biased the results towards an overestimation of the predictive value of colonized CSF-drain tips for secondary meningitis. The findings in this study may therefore hold less validity in a setting where all CSF-drains are cultured.

Nevertheless, our findings suggest that a positive culture from a CSF-drain tip will often be the first signal of secondary meningitis and that culture result may help guide the choice of antibiotic therapy. Therefore, direct communication of positive culture results by the microbiological laboratory to the treating physician should be common practice, and prophylactic antibiotic therapy should be considered, especially when a culture of an EVD tip yields *S. aureus* or Gram-negative bacteria.

References

1. Arabi Y, Memish ZA, Balkhy HH, Francis C, Ferayan A, Al Shimemeri A, et al: Ventriculostomy-associated infections: incidence and risk factors. **Am J Infect Control 2005**; 33:137-143

2. Conen A, Walti LN, Merlo A, Fluckiger U, Battegay M, Trampuz A: Characteristics and treatment outcome of cerebrospinal fluid shunt-associated infections in adults: a retrospective analysis over an 11-year period. **Clin Infect Dis 2008**; 47:73-82

3. Forgacs P, Geyer CA, Freidberg SR: Characterization of chemical meningitis after neurological surgery. **Clin Infect Dis 2001**; 32:179-185

4. Horan TC, Andrus M, Dudeck MA: CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. **Am J Infect Control 2008**; 36:309-332

5. Leverstein-van Hall MA, Hopmans TE, Berkelbach van der Sprenkel JW, Blok EM, van der Mark WA, Hanlo PW, et al: A bundle approach to reduce the incidence of external ventricular and lumbar drain-related infections. **J Neurosurgy 2010**; 112: 345-353

6. Lo CH, Spelman D, Bailey M, Cooper DJ, Rosenfeld JV, Brecknell JE: External ventricular drain infections are independent of drain duration: an argument against elective revision. **J Neurosurg 2007**; 106: 378-383

7. Lozier AP, Sciacca RR, Romagnoli MF, Connolly ES: Ventriculostomy-related infections: a critical review of the literature. **Neurosurgery 2002**; 51: 170-181

8. Ngo QN, Ranger A, Singh RN, Kornecki A, Seabrook JA, Fraser DD: External ventricular drains in pediatric patients. **Pediatr Crit Care Med 2009**; 10: 346-351

9. Schade RP, Schinkel J, Visser LG, van Dijk JM, Voormolen JH, Kuijper EJ: Bacterial meningitis caused by the use of ventricular or lumbar cerebrospinal fluid catheters. **J Neurosurg 2005**; 102: 229-234

10. Scheithauer S, Burgel U, Ryang YM, Haase G, Schiefer J, Koch S, et al: Prospective surveillance of drain associated meningitis/ventriculitis in a neurosurgery and neurological intensive care unit. **J Neurol Neurosurg Psychiatry 2009**; 80:1381-1385

11. van den Hoogen A, Brouwer MJ, Gerards LJ, Fleer A, Krediet TG: Removal of percutaneously inserted central venous catheters in neonates is associated with the occurrence of sepsis. **Acta Paediatr 2008**; 97:1250-1252

VI

Sensitivity and specificity of cerebrospinal fluid white blood cell counts for the diagnosis of secondary meningitis

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Submitted

Abstract

Object: Secondary meningitis is a frequent complication of neurosurgical procedures and is diagnosed upon clinical symptoms, cerebrospinal fluid (CSF) cell counts and biochemistry and microbiological culture results of CSF. White blood cell (WBC) counts constitute an important diagnostic criterion, but there is no consensus on the optimal WBC cut-off point or on the optimal adjustment for red blood cells (RBCs) in the CSF. We, therefore, aimed to determine (1) the optimal WBC cut-off point and (2) the optimal CSF WBC correction factor for RBCs for diagnosing secondary meningitis.

Methods: Data were extracted from the hospitals' microbiology laboratory management and information system. The diagnosis of secondary meningitis was based on the presence of CDC criteria and bacterial growth from CSF cultures. Control patients, with a similar risk of secondary meningitis were defined as patients with >4 negative CSF cultures without bacterial growth and without a clinical suspicion of secondary meningitis. Receiver operating characteristic curves were constructed to determine the optimal WBC cut-off point to diagnose secondary meningitis. The correction factor for RBCs was determined from CSF samples with > 1000 RBCs/mm^3, drawn from patients who did not suffer meningitis.

Results: 134 cases and 34 controls (with 227 control CSF samples) were included in the study. Median CSF WBC counts were 380 and 10 in patients with and without secondary meningitis, respectively. In patients with secondary meningitis, WBC counts were highest in episodes caused by Gram-negative micro-organisms. A sensitivity of 70% for secondary meningitis could be achieved with a specificity of approximately 80% applying a cut-off for CSF WBC counts of 90-100 WBCs/mm^3. A median of 1 WBC / 443 RBCs was found in patients with an initial CSF sample with > 1000 RBCs/mm^3; this ratio declined in subsequent CSF samples. Correction of the WBC count based on the RBC count changed the cut-off points, but did not improve the test characteristics.

Conclusions: Of the three parameters routinely determined in CSF, only WBC count was useful in diagnosing secondary meningitis. However, knowledge of the predictive value of different cut-off points is necessary for adequate evaluation of patients.

Introduction

Secondary meningitis is a frequent complication of neurosurgical procedures. [7] The interruption of the natural barriers facilitates invasive infection by micro-organisms which do not normally cause meningitis, ventriculitis or encephalitis. [4] The complication is reported to occur in 2 to 22% of patients with external cerebrospinal fluid (CSF) drainage, and in 1-18% of patients with internal shunts. [7] Data on development of secondary meningitis in neurosurgical patients without CSF-drains are not available. The attributable mortality has been estimated to be around 5%, [2, 4, 10] and the therapeutic consequences are major: treatment with two weeks of high-dose intravenous antibiotic therapy, and extraction or replacement of CSF-drains.

The diagnosis of secondary meningitis is based on neurological symptoms (in particular headache, neck stiffness, nausea and impaired consciousness), local and systemic signs of infection, CSF cell counts and biochemistry, and microbiological culture results. However, in patients at risk for secondary meningitis none of these diagnostic criteria are highly specific: frequently the patients' underlying diseases cause neurological symptoms; many patients suffer intracranial hemorrhage, which may cause fever; and CSF cell counts and CSF biochemistry may be altered as a result of intracranial blood.

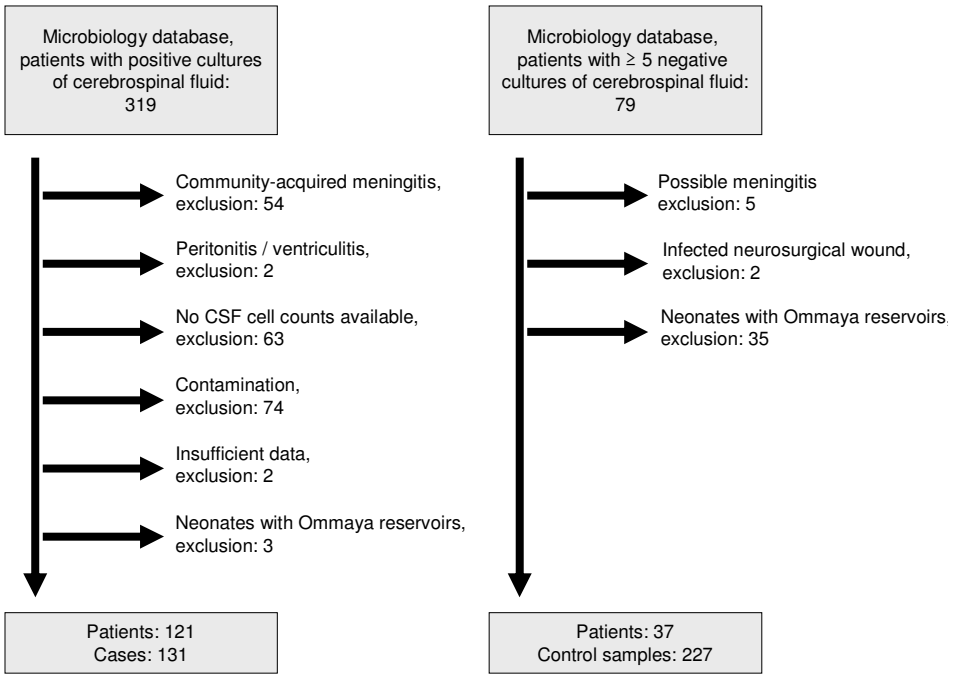
The best approximation of a gold standard for the diagnosis of secondary meningitis are CSF culture results. However, CSF samples may be contaminated with skin flora, or may remain falsely negative, due to antibiotic therapy. [2] Therefore, although the recommendations of the Infection in Neurosurgery Working Party of the British Society for Antimicrobial Chemotherapy state that antibiotic therapy should be discontinued in patients whose CSF-cultures are negative after three days of incubation, [6] in our experience, therapy is often continued for a full fourteen day in patients with an initial suspicion of secondary meningitis.

In particular CSF *pleiocytosis* is a clinical argument to start antibiotic treatment and/or to continue it when culture results have remained negative or are still pending. Antibiotic therapy is at times even administered in the absence of clinical symptoms, based solely on CSF WBC counts. However, although often a cut-off point of 10 WBCs/mm^3 is

employed to define pleiocytosis, this number is not evidence-based and cell counts in patients without meningitis may exceed this value. [9, 10] Furthermore, correction factors for CSF red blood cell (RBC) counts have not been established in patients with intracranial hemorrhage or in patients whose CSF punctures were traumatic, and it is unclear whether such a correction factor should be static or whether it should be adjusted for the time since hemorrhage.

The aims of this study were, therefore, threefold: (1) To determine the optimal breakpoints for CSF WBC counts in the diagnosis of secondary meningitis. (2) To determine the optimal WBC-correction factor for RBCs in the CSF. (3) To determine whether the CSF RBC/WBC ratio changes over time.

Figure 1: flowchart of inclusion of cases and controls



Patients and methods

Setting and databases

The study was performed at the UMC Utrecht, a 1,042-bed academic teaching hospital in the centre of the Netherlands, with annually about 28,000 clinical and 15,000 day-care hospitalizations and 334,000 outpatient visits. Data were used from the hospital's electronic patient charts, from the consultation files of the clinical microbiology and infectious diseases department, from the microbiology laboratory management and information system and from the Utrecht Patient Oriented Database (UPOD), obtained between January 1st 2005 and January 1st 2010. UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders and laboratory tests for all patients treated at the University Medical Center Utrecht (UMC Utrecht) since 2004. UPOD data acquisition and management is in accordance with current regulations concerning privacy and ethics. The structure and content of UPOD have been described in more detail elsewhere. [1] The search strategy included identification of all patients with positive CSF cultures (to identify patients with secondary meningitis) and all patients with at least five CSF cultures in the microbiology laboratory management and information system (to identify high-risk with no evidence of secondary meningitis).

Patients

Secondary meningitis was defined in accordance with the CDC-guidelines, [5] but with the prerequisite that CSF-cultures were positive in order to maximize the likelihood of true infection. Secondary meningitis was, thus, defined as: (1) one or more positive CSF-cultures; AND (2) a diagnosis of secondary meningitis made by the attending physician; AND (3) treatment of the patient for secondary meningitis according to the hospital guidelines. Patients with ventriculo-peritoneal drains (VPDs) or lumbo-peritoneal drains (LPDs) were excluded if they suffered from peritonitis, as were neonates with Ommaya reservoirs, and patients for whom no CSF WBC and RBC counts were available from the day the first culture positive CSF sample was drawn.

Patients with a minimum of five CSF-cultures, and without bacterial growth from any of these cultures were identified and considered to represent a population at high risk for secondary meningitis and with a high likelihood of true absence of infection. Patients with any positive CSF-culture were excluded as controls, even if the culture was deemed contamination, as were neonates with Ommaya reservoirs, patients who received treatment for secondary meningitis according to the hospital guidelines, and patients with haematological malignancies with central nervous system (CNS) involvement. All samples of these patients were included.

CSF cell count

After lumbar puncture the CSF samples were collected in polypropylene tubes and within 30 minutes delivered to the laboratory, cell counting was performed within 60 minutes after puncture. Cells in clear colourless CSF samples were counted in a microscopic counting chamber (Fuchs-Rosenthal) after staining with methyl-violet. Cells in cloudy, purulent or bloody samples were counted with a hematology analyser (Abbott Cell-Dyn Sapphire, Abbott Diagnostics, Santa Clara, CA, USA). [8, 12] This hematology analyser uses a multiangle polarization scatter separation (MAPSS) technology in the optical channel (white blood cell count), in combination with a second channel with impedance count (red blood cell count). In preparation for CSF cell counting, the hematology analyser was flushed to obtain background cell counts of $< 0.01\ 10^9$/L (white blood cells) and $< 0.01\ 10^{12}$/L (red bloods cells). The sample was measured in duplicate in the "white blood cell extended count mode". For practical (mathematical) purposes, a WBC count of 0 was counted as 0.5.

Bacterial culture

CSF was inoculated on blood agar and chocolate agar and incubated for two days at 35º C in 5% CO_2, and on brucella blood agar and in brain-heart XV broth and incubated for seven days anaerobically at 35º C.

Determination of the CSF RBC/WBC ratio

The RBC / WBC ratio – putatively the correction factor for RBCs in CSF – was calculated from the patients with (1) a CSF sample with >1000 RBCs/mm3, and (2) two additional samples taken within intervals of 3 to 14 days (between 1st and 2nd, and between 2nd and 3rd sample). Patients were included only once, based on the first sample with > 1000 RBCs / mm^3 in the study period. Patients with a diagnosis of meningitis or haematological malignancy with CNS involvement were excluded. Putatively, the ratio between WBC and RBC counts of these patients would determine the correction factor for WBC counts in patients with traumatic CSF-punctures or intracranial bleeding. A cut-off value of 1000 RBCs/mm^3 was chosen, as it was not to be expected that lower amounts of blood in the CSF-sample would significantly alter the WBC count.

Data analysis

Dichotomous variables were analyzed by Chi-square and Fisher's exact test where appropriate. Continuous variables were analyzed by Mann Whitney U test for independent groups, and or by Wilcoxon matched pairs test for the progression of RBC/WBC ratios. Receiver operating characteristic (ROC) curves were constructed to establish the optimal cut-off points for sensitivity and specificity. Correction factors of 1 WBC/1000 RBCs, 1 WBC/500 RBCs and 1 WBC/100 RBCs were analyzed.

Statistical analysis was performed with GraphPad Prism version 5.03. Sensitivity / specificity analysis was performed for the group as a whole, and with exclusion of neonates.

Table 1: Characteristics of patients and controls	Patients with secondary meningitis (n=131)	Controls (n=37 patients, 227 samples)	P-value
Age, median (IQR)	50 (18-64)	47 (10-66)	0.56
Male sex	62 (47%)	15 (41%)	0.58
Drainage[1]			
External ventricular drain	66 (50%)	23 (62%)	0.26
External lumbar drain	18 (14%)	11 (30%)	0.03
Ventriculo-peritoneal drain	30 (23%)	13 (35%)	0.14
Lumbo-peritoneal drain	0	4 (11%)	0.002
Other[2]	2 (1.5%)	0	1.0
No drain	15 (11%)	2 (5.4%)	0.37
CSF parameters			
Red blood cell count, median (IQR)	528 (43-9941)	304 (26-3413)	0.07
White blood cell count, median (IQR)	380 (34-2385)	10 (3-52)	<0.0001
Glucose (in mmol/L), median (IQR)	3.1 (2.5-4.0)	3.4 (2.5-4.2)	0.26
Total protein (in g/L), median (IQR)	0.96 (0.53-2.3)	0.66 (0.37-1.13)	0.0001
Culture results			
S. aureus	35 (27%)	-	-
Gram-negative bacteria[3]	22 (17%)	-	-
Coagulase-negative staphylococci	52 (40%)	-	-
Other Gram-positive bacteria[4]	22 (17%)	-	-

IQR: interquartile range; [1]the number of drains exceeded the number of controls, as some controls had different drains in the course of their treatment; [2]one Rickham drain and one Jackson-Pratt drain; [3]Gram-negative isolates: 7 *Enterobacter cloacae*, 4 *Serratia marcescens*, 3 *Escherichia coli*, 3 Acinetobacter sp., 2 *Pseudomonas aeruginosa*, 1 *Klebsiella pneumoniae*, 1 *Citrobacter koseri*, 1 *Flavimonas johnsonii*; [4]other Gram-positive isolates: 5 Enterococcus sp., 5 hemolytic streptococci, 1 other streptococci, 4 *Bacillus cereus*, 3 Propionibacterium sp., 3 Corynebacterium sp., 2 Gram-positive rods not determined.

Results

Of all 319 patients with microbiological growth in CSF, 198 patients were excluded, because of a diagnosis of community-acquired/neonatal meningitis (n=54) or internal CSF-drain associated peritonitis/ventriculitis (n=2), because no CSF cell counts were available (n=63, often contaminated samples sent in for post-mortem culture), or because cultures were deemed to represent contamination (n=74). In two patients insufficient data were available to determine whether secondary meningitis was present, and three patients were neonates with Ommaya reservoirs. Finally a total of 121 patients were included; eight patients experienced two separate episodes of secondary meningitis and one patient experienced three separate episodes, making a total of 131 episodes (Figure 1). Patient data and culture results are depicted in Table 1.

Median CSF WBC count in patients with meningitis was 360/mm^3, and WBC counts were highest in patients with meningitis caused by Gram-negative bacteria (median 1706, IQR 631-7735; p=0.0007 when compared to all other bacteria and p=0.031 when compared to *S. aureus*) (Figure 2). CSF WBC counts did not differ statistically significant between infections caused by *S. aureus*, CoNS and other Gram-positive micro-organisms.

In all, 79 patients had >4 negative CSF cultures; 35 were neonates with Ommaya reservoirs, in five meningitis could not be excluded and two had infections of their neurosurgical wounds, leaving 37 patients (227 samples) for analysis (Table 1 and Figure 1).

Sensitivity and specificity of CSF parameters

The area under the curve (AUC) of the ROC curve for diagnosing secondary meningitis was 0.81 (95% CI 0.76-0.87) for the crude WBC counts, with 93 WBCs/mm^3 as the most optimal cut-off point (70% sensitivity and 78% specificity). Adjustment of WBC for the presence of RBC did not improve the AUCs, which were 0.81 (95% CI 0.76-0.86), 0.81 (95% CI 0.76-0.86), 0.78 (95% CI 0.73-0.83) for 1 WBC/1000 RBCs, 1 WBC/500 RBCs and 1 WBC/100 RBCs, respectively. Naturally, optimal cut-off points did change and these were 79 WBCs/mm^3 (70% sensitivity and 79% specificity), 69 WBCs/mm^3 (70% sensitivity and 81%

specificity), and 22.5 WBCs/mm^3 (70% sensitivity and 79% specificity) for 1 WBC/1000 RBCs, 1 WBC/500 RBCs and 1 WBC/100 RBCs, respectively (Figure 4 and Table 2).

Total protein was higher in patients with secondary meningitis (0.96 g/L vs 0.66 g/L in patients without infection (p=0.001)), but the AUC of this parameter was only 0.62 (95% CI 0.56-0.69).

Determination of the CSF RBC/WBC ratio

There were 128 patients with three CSF samples available of which the first sample had >1000 WBCs/ mm^3. The median RBC/WBC ratios declined significantly in time from 254 (1st sample) to 137 (2nd sample) to 90 (3rd sample) (Figure 3).

Table 2: Receiver operating characteristics for secondary meningitis, with as a control group patients with >4 negative cerebrospinal fluid cultures (positives n=131 samples, controls n=227 samples)

Formula	Sensitivity	Specificity	Cut-off (WBCs/mm^3)	Area under ROC curve
WBCs	70%	78%	93	0.81
	80%	60%	18	
	90%	42%	7	
WBCs – RBCs/1000	70%	79%	79	0.78
	80%	61%	16.5	
	90%	44%	5.9	
WBCs – RBCs/500	70%	81%	69	0.81
	80%	63%	15.4	
	90%	44%	4.7	
WBCs – RBCs/100	70%	79%	22.5	0.81
	80%	60%	4.4	
	90%	18%	-23	

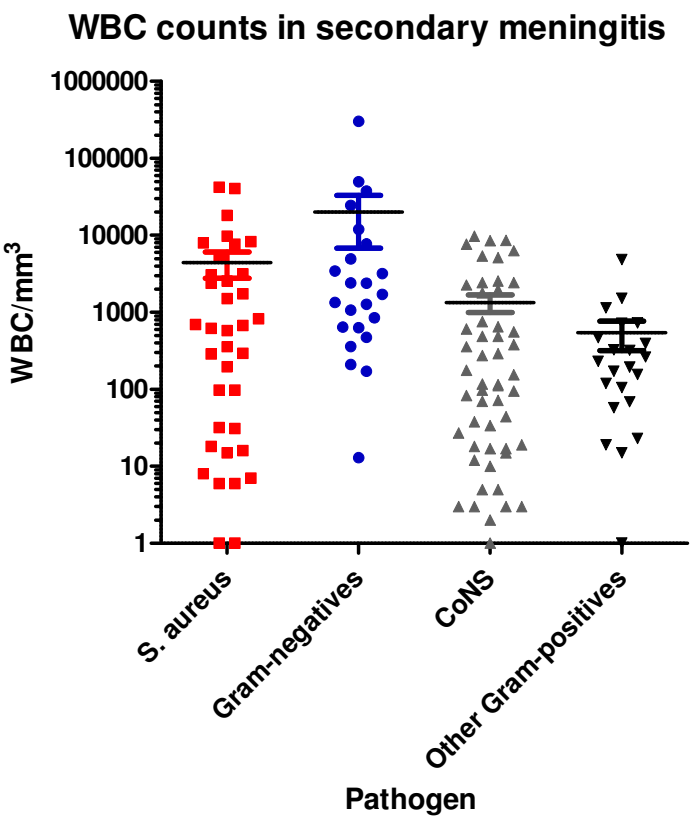
Discussion

In the present study with 131 episodes of "definite" presence and 227 episodes of "definite" absence of secondary meningitis, a cut-off value of 93 WBCs/mm^3 was associated with 70% sensitivity and 78% specificity for establishing the diagnosis. The AUC of the ROC for the crude CSF WBC was 0.81 (95% CI 0.76-0.87) and this value did not improve after adjustment for the numbers of RBC in CSF. CSF glucose and CSF total protein were not useful in diagnosing secondary meningitis.

Previous studies on this subject applied different correction factors for RBC counts, varying from no correction, [2, 11] to 1 WBC / 1000 RBCs, [10] to 1 WBC / 100 RBCs. [3] Cut-off points for pleiocytosis have been suggested at > 5 WBCs / mm^3, [2] > 10 WBCs/mm^3, [7] and >50 WBCs/mm^3. [9] In two prospective studies in which CSF samples were evaluated daily in patients with external drains, correlations were found between secondary meningitis (defined as a positive CSF culture and a clinical diagnosis) and CSF WBC counts, but useful cut-off points could not be identified. [9, 10]

The difference in predictive value of WBC counts between our study and these two previous studies may have resulted from the different approach to the diagnosis of secondary meningitis. In both previous studies CSF samples were obtained routinely on a daily basis. In one study all meningitis patients appeared to have fever, but only 32% had accompanying neurological signs or symptoms. [10] Because of the low specificity of fever for secondary meningitis in this patient group, it is possible that patients were misclassified as secondary meningitis based on contaminated CSF. In the other study neurological symptoms were not specified. [9] In the present study, CSF samples were – in principle – only drawn when secondary meningitis was suspected. Therefore, the prior chances of meningitis were higher, reducing the relative contribution of false-positive findings. Also in other studies which did not draw daily CSF samples, but included patients based on clinical findings, the WBC counts of meningitis patients were generally higher than in the studies by Schade e.a. and Pfisterer e.a.. [2, 3, 11] However, none of these studies compared the meningitis patients to a control group, and they did not determine sensitivity, specificity or predictive values.

Figure 2A



Legend figure 3 (opposite page): Medians (bars), interquartile ranges (boxes), and total range (whiskers) of red blood cell (RBC) / white blood cell (WBC) ratio's in 128 patients with > 1000 RBCs/mm^3 in cerebrospinal fluid (CSF), a minimum of three included samples, and no diagnosis of meningitis. Statistical analysis was performed with the Wilcoxon matched pairs test.

Figure 2B

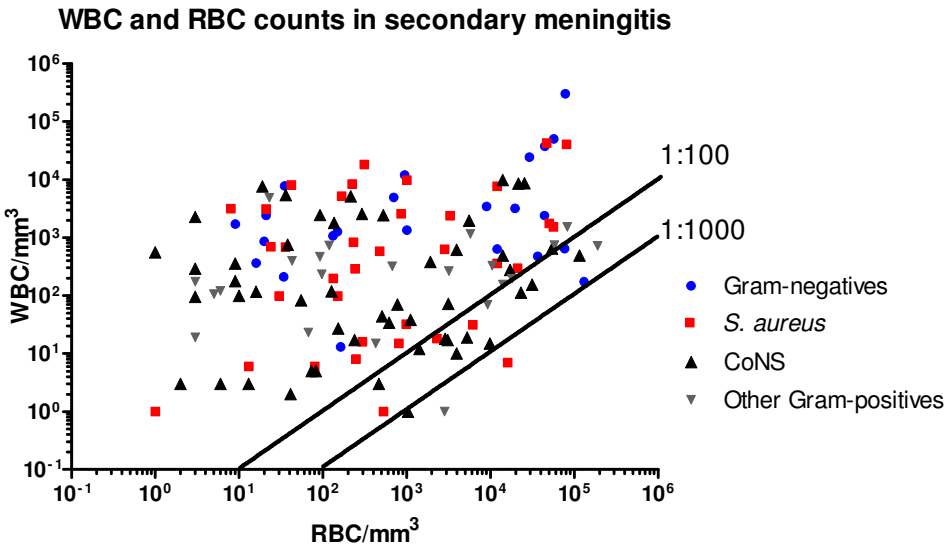
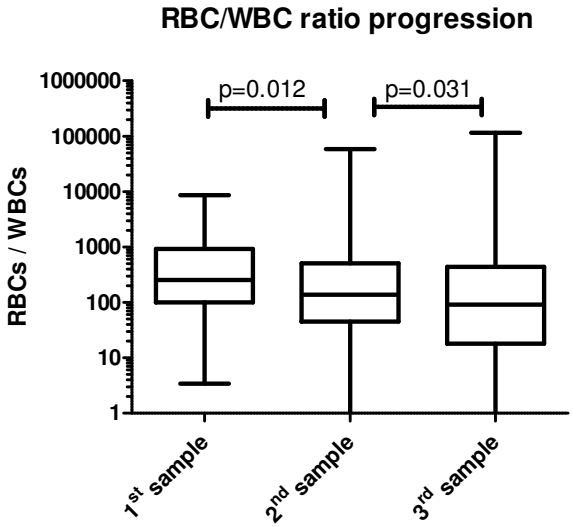


Figure 3:



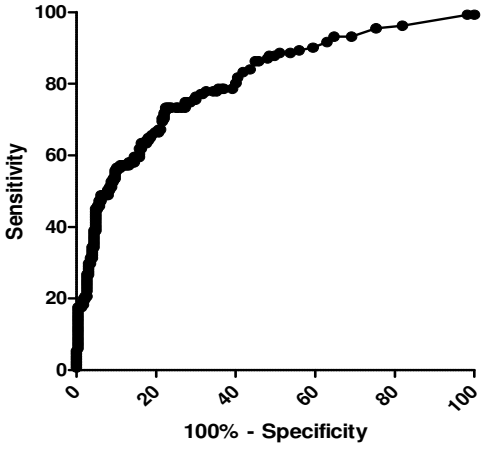
Sixteen patients suffered secondary meningitis caused by Gram-negative rods. WBC counts in these infections were significantly higher than with other pathogens. If this finding is confirmed by others, it may suggest a different approach to the treatment of "culture-negative secondary meningitis" in patients without pleiocytosis (>100 WBCs/mm^3). When cultures remain negative after 48-72 hours, it may be justified to halt Gram-negative coverage and treat with vancomycin monotherapy.

This study, as all other previous studies in this area, suffers some limitations. First and foremost, there is no gold standard for the diagnosis. We tried to circumvent this problem by maximizing the likelihood of true presence or absence of infection. Therefore, bacterial growth from CSF was a prerequisite for the diagnosis and absence of any bacterial growth for the other category. This implied that five patients with high WBC counts and negative cultures, that had been labelled "secondary meningitis" by attending physicians were excluded from the current analysis

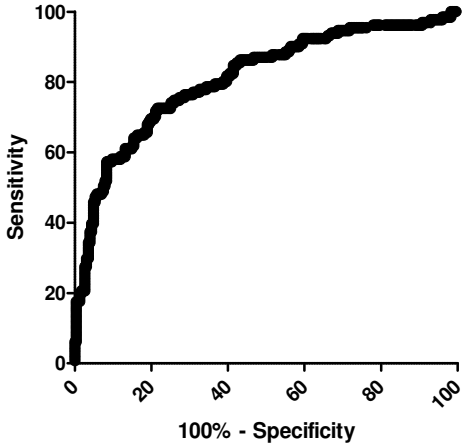
In the current setting at our institution, empiric antibiotic therapy is readily initiated when secondary meningitis is suspected. The results from this study do not provide a clear-cut breakpoint to single out the patients with such an infection. They do, however, provide insight into the predictive values of CSF parameters, and will support clinicians in deciding whether or not to start or continue treatment when the diagnosis is dubious, or frankly improbable.

Figures 4A-D (opposite page): Receiver operation characteristics (ROC) curves for white blood cell (WBC) counts in the diagnosis of secondary meningitis, uncorrected and corrected for red blood cell (RBC) counts.

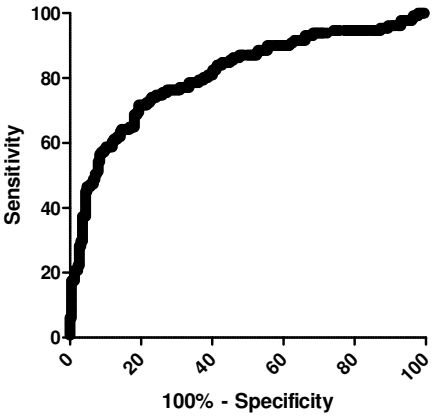
4A: Uncorrected WBC counts



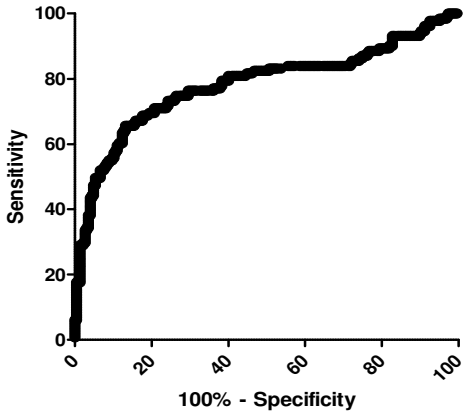
4B: WBC counts – (RBC count / 1000)



4C: WBC counts – (RBC count / 500)



4D: WBC counts – (RBC count / 100)



References

1. ten Berg MJ, Huisman A, van den Bemt PM, Schobben AF, Egberts AC, van Solinge WW: Linking laboratory and medication data: new oppportunities for pharmacoepidimioloical research. **Clin Chem Lab Med 45**: 13-19, 2007

2. Conen A, Walti LN, Merlo A, Fluckiger U, Battegay M, Trampuz A: Characteristics and treatment outcome of cerebrospinal fluid shunt-associated infections in adults: a retrospective analysis over an 11-year period. **Clin Infect Dis 47**:73-82, 2008

3. Forgacs P, Geyer CA, Freidberg SR: Characterization of chemical meningitis after neurological surgery. **Clin Infect Dis 32**:179-185, 2001

4. 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**: Epub ahead of print, Jul 2, 2010

5. Horan TC, Andrus M, Dudeck MA: CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. **Am J Infect Control 36**:309-332, 2008

6. Infection in Neurosurgery Working Party of the British Society for Antimicrobial Chemotherapy: The management of neurosurgical patients with postoperative bacterial or aseptic meningitis or external ventricular drain-associated ventriculitis. **Br J Neurosurg 14**:7-12, 2000

7. Leverstein-van Hall MA, Hopmans TE, Berkelbach van der Sprenkel JW, Blok EM, van der Mark WA, Hanlo PW, et al: A bundle approach to reduce the incidence of external ventricular and lumbar drain-related infections. **J Neurosurgy 112**: 345-353, 2010

8. Müller R, Mellors I, Johannessen B, Aarsand AK, Kiefer P, Hardy J, et al: European multi-center evaluation of the Abbott Cell-Dyn sapphire hematology analyzer. **Lab Hematol 12**: 15-31, 2006

9. Pfisterer W, Mühlbauer M, Czech T, Reinprecht A: Early diagnosis of external ventricular drainage infection: results of a prospective study. **J Neurol Neurosurg Psychiatry 74**: 929-932, 2003

10. Schade RP, Schinkel J, Visser LG, van Dijk JM, Voormolen JH, Kuijper EJ: Bacterial meningitis caused by the use of ventricular or lumbar cerebrospinal fluid catheters. **J Neurosurg 102**:229-234, 2005

11. Scheithauer S, Bürgel U, Ryang YM, Haase G, Schiefer J, Koch S, et al: Prospective surveillance of drain associated meningitis/ventriculitis in a neurosurgery and neurological intensive care unit**. J Neurol Neurosurg Psychiatry 80**:1381-1385, 2009

12. De Smet D, Van Moer G, Martens GA, Nanos N, Smet L, Jochmans K, et al: Use of the Cell-Dyn Sapphire hematology analyzer for automated counting of blood cells in body fluids. **Am J Clin Pathol 133**:291-299, 2010

VIIa

Bacteremic complications of intravascular catheters colonized with *Staphylococcus aureus*.

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Abstract

Patients with *Staphylococcus aureus* colonization of an intravascular catheter, but without demonstrated bacteremia within 24 hours of IV-catheter removal, had a 24% (12/49) chance of subsequent *S. aureus* bacteremia if they did not receive immediate anti-staphylococcal antibiotics. Treatment within 24 hours of IV-catheter removal led to a 83% reduction of subsequent bacteremia.

Introduction

Whether "patients with positive results of catheter cultures (but with negative blood culture results) and no other obvious site of infection need to be treated with antibiotics", was defined as an area for future research in the Infectious Diseases Society of America (IDSA) Guidelines for Management of Intravascular Catheter-Related Infections.[1]

Intravascular catheter (IV-catheter) tip colonization with *Staphylococcus aureus* is strongly associated with concomitant bacteremia,[2,3] and therefore an IV-catheter tip culture yielding *S. aureus* is generally regarded as an alarming signal. However, evidence guiding the appropriate medical care in such circumstances is lacking. Several studies evaluated the consequences of infected or colonized IV-catheters in patients *with* concomitant bacteremia. To our knowledge, the outcome of patients who had IV-catheter colonization by *S. aureus without* concomitant bacteremia, has been assessed only once.[4]

As there was no protocol for guiding patient management in patients with *S. aureus* positive IV-catheter tip cultures in the absence of concomitant bacteremia, practices differed widely between treating physicians; furthermore, blood cultures were not consistently taken in all patients. We therefore retrospectively assessed the incidence of bacteremic complications in patients with *S. aureus*-colonized catheter tips and absence of concomittant bacteremia, and the effects of antibiotic therapy hereon.

Patients and methods

Patient inclusion:

Patients with *S. aureus* cultured from intravascular catheters between 1 June 2001 and 1 October 2006 were identified in the database of the bacteriology department of the University Medical Center Utrecht (a tertiary care hospital with 1,024 beds). From the same database it was determined whether blood cultures had been obtained from these patients in the 24 hours before and after IV-catheter removal, and whether the patients had had any

positive blood culture with *S. aureus* in the six months before and after catheter removal. Patients with positive blood cultures with *S. aureus* in the period from 48 hours before, to 24 hours after IV-catheter removal were excluded from the analysis. Risk factors were recorded from the patient charts and all patients were followed for a period of six months from the day of IV-catheter removal.

Definitions:

A subsequent bacteremic complication was defined as *S. aureus* bacteremia (SAB) with an isolate with a susceptibility pattern identical to that of the isolate from the catheter tip, demonstrated > 24 hours after removal of the intravascular catheter. Treatment of *S. aureus* was defined as any therapy (be it intravenous or oral) for which the cultured *S. aureus* strain was tested susceptible, started within 24 hours of removal of the intravascular catheter and continued for a minimum of three days. An IV-catheter exit site infection was defined as redness, swelling, purulence or tenderness of the IV-catheter exit site.

Bacteriology:

Catheter tips were processed as described previously.[5] Cultures yielding 15 colony forming units (cfu's) or more were considered positive. Blood cultures were incubated for a minimum of 5 days at 35˚ C.

Data analysis:

Risk factors for subsequent *S. aureus* bacteremic complications were analyzed by Fisher exact test and chi-square for nominal variables, and by Mann-Whitney test for continuous variables. Statistically significant risk factors were subsequently analyzed by multivariate logistic regression. Furthermore, the association between antibiotic treatment, which was putatively the main protective factor, and signs and symptoms of infection were determined.[6]

Results

In the 63-month-period, 184 patients had intravascular catheter tips positive with *S. aureus*, of which 99 patients (54%) had no evidence of SAB up to 24 hours after removal. The cultured catheter tips came from 11 tunneled catheters, 21 subclavian central venous catheters (CVC), 24 jugular CVC, 18 femoral CVC, 4 umbilical CVC, 2 peripherally inserted central catheters (PICC), and 8 arterial catheters; the insertion site of 11 CVC could not be retrieved. The average time of catheter presence was 24.2 and 17.4 days in patients with and without subsequent SAB, respectively (Table 1). These relatively long periods were attributable to the tunneled catheters (average duration of 67 days); this period was shorter for CVC and arterial catheters, 11.6 days and 8.3 days, respectively. Half of the patients (n=50) received anti-staphylococcal treatment for an average of 11.1 days (range 3 – 21 days); three of these patients received only oral therapy.

Fourteen patients suffered subsequent SAB at an average of 6.7 days after removal of the intravascular catheter (median 3 days, range 2 – 25 days). Risk factors and characteristics are displayed in table 1. Twelve out of 49 patients (24%) who did not receive antibiotic treatment developed subsequent SAB, which contributed directly to death in two patients (4%): The first case was a 67-year-old woman who underwent resection of a hepatocellular carcinoma; she died of fulminant *S. aureus* sepsis which manifested itself three days after removal of the IV-catheter. The second was a 57-year-old woman who had been admitted for abdominal surgery; she was discharged the same day her IV-catheter was removed, but two days later she was re-admitted to the hospital with *S. aureus* sepsis to which she succumbed. Furthermore, in a third patient who suffered from terminal heart failure it is likely that *S. aureus* bacteremia aggravated the underlying disease, ultimately leading to death. Two of the 50 treated patients (4%) had subsequent complications: a case of thrombophlebitis and a case of IV-catheter-associated SAB. Other complications are displayed in table 2. Thus, antibiotic treatment had a protective effect for subsequent SAB (OR 0.13; 95%-CI: 0.02 – 0.61); this result was significant in both the univariate and multivariate analyses. The only other independent risk factor for subsequent SAB was

corticosteroid use (OR 6.1; 95%-CI: 1.8 – 20.4). Because of small numbers it was not possible to evaluate associations between different catheter types and subsequent SAB. There were no cases of subsequent SAB in the 8 patients with colonized arterial catheters.

6-month survival was 64.3% in the group with subsequent SAB and 84.7% in the group without *S. aureus* bacteremic complications (p=0.13). 6-month survival was 84.0% in the patients who received antibiotic treatment, and 79.6% in patients who did not (p=0.32).

The presence of systemic inflammatory response syndrome (SIRS)[6] was associated with antibiotic treatment (Table 3), but not with documented signs of exit site infection.

Legend table 1 (opposite page): COPD, chronic obstructive pulmonary disease; SD, standard deviation; [1]documented symptoms of IV-catheter exit site infection: dysfunctioning n=3, redness n=25, purulence n=23, pain n=9 (overlap was found between the different symptoms); [2]documented symptoms of IV-catheter exit site infection: disfunctioning 0, redness 4, purulence 6, pain 2 (overlap was found between the different symptoms); [3]SIRS, systemic inflammatory response syndrome (two or more of the following: temperature >38° C or <36° C; white blood cell count > 12.000 cells/mm^3 or >10% immature forms; heart rate > 90 beats/minute; respiratory rate > 20 breaths/minute); [4]culture with S. aureus from a site other than the IV-catheter or the IV-catheter exit site within 24 hrs of IV-catheter removal (in the group without subsequent SAB this consisted of: respiratory tract culture n=11, CAPD-fluid n=1, material from abdominal wound drain n=1, wound infection n=1; in the group with subsequent SAB one patient (nr 4 in table 2) had a positive sputum culture).

Table 1: Risk factors for *Staphylococcus aureus*-related bacteremic complications among 99 patients with *S. aureus* isolated from intravascular-catheter tips

Risk factor	Cases without subsequent SAB (n=85)	Cases with subsequent SAB (n=14)	p-value	OR (95%-confidence interval)
Age (Average)	37.7 years	44.8 years	0.45	
Male gender	45 (53%)	8 (57%)	0.77	
Co-morbid conditions				
Underlying malignancy	19 (22%)	4 (29%)	0.73	
Diabetes mellitus	6 (7%)	0	0.59	
Haemodialysis	1 (1%)	0	0.68	
Mechanic ventilation	30 (35%)	5 (36%)	0.93	
COPD	4 (5%)	2 (14%)	0.16	
Average duration IV-catheter in situ (SD)	17.4 days (+/- 28.6)	24.2 days (+/- 20.9)	0.31	
Average length of stay before removal of IV-catheter (SD)	23.6 days (+/- 28.4)	47.3 days (+/- 50.8)	0.08	
Use of IV-catheter for:				
Total parenteral nutrition	20 (23%)	6 (43%)	0.10	
Inotropic therapy	11 (13%)	0	0.60	
Chemotherapy	8 (9%)	2 (14%)	0.63	
IV-catheter exit site infection	37[1] (44%)	8[2] (57%)	0.34	
SIRS[3]	35 (41%)	3 (21%)	0.24	
Alternative site of infection[4]	14 (16%)	1 (7%)	0.69	
Immunosuppressive therapy	9 (11%)	3 (21%)	0.37	
Systemic corticosteroid use	12 (14%)	7 (50%)	0.005	6.1 (1.8 – 20.4)
Antibiotic treatment	48 (56%)	2 (14%)	0.003	0.13 (0.02 – 0.61)

Table 2: Bacteremic complications 2 days or more after

	Age and gender	Underlying pathology	Type of intravascular catheter	Interval[2] (days)	Therapy within 24 hrs[3]
1	34M	Complicated femur fracture	PICC	2	flucloxacillin
2	53F	Polyarthritis nodosa and bowel perforations	Hickman-catheter	2	none
3	62M	Hypopharynx-carcinoma	CVC, exit site not documented	2	none
4	67F	Necrotising pancreatitis	CVC subclavian vein	2	none
5	75F	Renal transplant patient, COPD, admitted with ileus	CVC subclavian vein	2	none
6	83M	Bradycardia, out-of-hospital reanimation, COPD	CVC femoral vein	2	none
7	48M	Non-Hodgkin lymphoma	CVC femoral vein	3	none
8	67F	Hepatocellular carcinoma, resection of liver segments	CVC subclavian vein	3	none
9	12M	SCT; graft vs host disease	PICC	5	none
10	0.5F	Lunghypoplasia	CVC femoral vein	8	none
11	7F	SCT, graft vs host disease	Hickman-catheter	11	none
12	12M	Colitis ulcerosa.	Hickman-catheter	13	none
13	46M	Respiratory insufficiency of unknown etiology	CVC subclavian vein	13	none
14	78M	Multitrauma patient	CVC femoral vein	25	meropenem

[1]Gender: M = male, F = female; [2]interval (in days) between removal of the IV-catheter and demonstrated bacteremia; [3]therapy initiated within 24 hours of IV-catheter removal; [4]documented signs of IV-catheter exit site infection (disfunctioning, redness, pain, purulence, swelling)

removal of an intravascular catheter colonized with *S. aureus*

	Exit site infection[4]	Cortico-steroid use	Description of the subsequent bacteremic episode
1	Yes	No	Thrombophlebitis at time of IV-catheter removal. Infected thrombus diagnosed after 48 hours. Surgical removal of the thrombus.
2	Yes	Yes	Hickman catheter replaced because of purulence exit-site. Subsequent SAB and removal of newly placed Hickman catheter.
3	No	No	SAB of unknown origin. Treatment initiated on 2nd day after CVC removal. No clinical deterioration in the meantime.
4	No	No	SAB of unknown origin. Treatment initiated on 2nd day after CVC removal. No clinical deterioration in the meantime.
5	Yes	Yes	Discharged the day of CVC removal. Re-admitted two days later with *S. aureus* septic shock and thrombosis of the sinus cavernosus. Patient succumbed within 24 hours.
6	No	Yes	Heart failure, possibly aggravated by *S. aureus* bacteremia. Death three days after IV-catheter removal.
7	Yes	No	Abscess of left elbow.
8	Yes	No	Three days after removal of the CVC *S. aureus* septic shock, multi-organ failure, death.
9	Yes	Yes	Arthritis of right elbow.
10	Yes	No	SAB of unknown origin with deterioration of respiratory function and SIRS.
11	Yes	Yes	Infected port-a-cath, placed three days after removal of the Hickman-catheter.
12	No	Yes	Osteomyelitis right ankle and knee two weeks after removal of the Hickman-catheter.
13	No	Yes	SAB of unknown origin and SIRS.
14	No	No	Colonization of removed femoral CVC. Treatment with meropenem and later ciprofloxacin, while subclavian CVC remained in situ. SAB after cessation of antibiotic therapy.

CA-SAB: catheter-associated Staphylococcus aureus bacteremia; SIRS: systemic inflammatory response syndrome; COPD: chronic obstructive pulmonary disease; CVC: central venous catheter; PICC: peripherally inserted central venous catheter; SCT: stem cell transplantation

Discussion

Colonization of intravascular catheters with *S. aureus* was associated with a 14% incidence of subsequent bacteremic complications and these complications occurred more frequently in patients not receiving anti-staphylococcal antibiotics within 24 hours after catheter removal (24% versus 4%). Antibiotic treatment was, thus, associated with a 87% lower risk of subsequent SAB. In a previous study 39% of patients with *S. aureus* isolated from catheter tips and without concomitant bacteremia developed SAB within a period of 12 weeks, and antibiotic therapy was associated with a 91% reduction of subsequent SAB.[4] The results of these two studies strongly suggest that antibiotic treatment of all patients with *S. aureus*-positive IV-catheter tips could prevent a considerable number of bacteremic complications.

We considered antibiotic treatment as immediate when initiated within 24 hours after IV-catheter removal. In other studies evaluating outcomes of SAB a period 48 hours after documentation of bacteremia or tip colonization has been used.[4,7] However, IV-catheter tip colonization with *S. aureus* can be demonstrated within 24 hours in most well-equipped bacteriological laboratories, and a 48 hour period, therefore, does not reflect standard care.[8] Our conclusions, though, would not change if we had considered antibiotic treatment initiated within 48 hours as immediate treatment.

The association between catheter tip colonization and subsequent SAB (as well as the observed protective effects of antibiotics), actually, may have been underestimated in our study. The decision of physicians to initiate antibiotic treatment or not, was not protocolized. Prescription of antibiotics was strongly associated with the presence of signs and symptoms of (systemic) infection; therefore, those patients probably at highest risk for subsequent SAB already received treatment more frequently, introducing a bias against the observed correlation.

Because of its retrospective design this study suffers from several limitations: blood cultures were not taken in a protocolized manner, and *S. aureus* strains were not available for typing. Since culturing catheter tips was based on clinical decision-making, a selected subset of intravascular catheter tips was sent in for culturing, and the results may not be

extrapolated to a scenario in which all catheter tips are cultured. Finally, the number of patients included was small compared to the number of risk factors evaluated, compromizing the power of both the univariate and the multivariate analysis.

Apart from not receiving antibiotics, only corticosteroid use was significantly associated with subsequent SAB. For other potential risk factors, such as total parenteral nutrition, type of IV-catheter, and the length of catheter presence before removal, only trends towards an association with subsequent SAB could be demonstrated. Larger prospective studies are needed to identify risk factors for subsequent SAB, and to establish the optimal duration of treatment; for the moment however, in the absence of randomized prospective trials, the best available evidence supports rapid antibiotic treatment of all patients with *S. aureus*-positive IV-catheter tips.

Table 3: Association between antibiotic therapy and signs of infection in patients with *Staphylococcus aureus* colonization of an intravascular catheter

Characteristic	Antibiotic therapy (n=50)	No antibiotic therapy (n=49)	p-value
SIRS[1]	28 (56%)	10 (20%)	<0.001
IV-catheter exit site infection	23 (46%)	22 (44%)	0.912

[1]*SIRS, systemic inflammatory response syndrome (two or more of the following: temperature >38° C or <36° C; white blood cell count > 12.000 cells/mm^3 or >10% immature forms; heart rate > 90 beats/minute; respiratory rate > 20 breaths/minute)*

References

1. Mermel LA, Farr BM, Sherertz RJ, et al. Guidelines for the management of intravascular catheter-related infections. **Clin Infect Dis 2001**; 32:1249-1272.

2. Peacock SJ, Eddleston M, Emptage A, King A, Crook DW. Positive intravenous line tip cultures as predictors of bacteremia. **J Hosp Infect 1998**; 40:35-38.

3. Siegman-Igra Y, Anglim AM, Shapiro DE, Adal KA, Strain BA, Farr BM. Diagnosis of vascular catheter-related bloodstream infection: a meta-analysis. **J Clin Microbiol 1997**; 35:928-936.

4. Ruhe JJ, Menon A. Clinical significance of isolated *Staphylococcus aureus* central venous catheter tip cultures. **Clin Microbiol Infect 2006**; 12:933-936.

5. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. **N Engl J Med 1977**; 296:1305-1309.

6. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. **Crit Care Med 1992**; 20:864-874.

7. Fowler VG, Jr., Olsen MK, Corey GR, et al. Clinical identifiers of complicated Staphylococcus aureus bacteremia. **Arch Intern Med 2003**; 163:2066-2072.

8. Bannerman TL. Staphylococcus, micrococcus, and other catalase-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. **Manual of Clinical Microbiology 2003**; 8th ed.:384-404.

VIIb

Staphylococcus aureus bacteremia and sepsis is preventable in patients with *Staphylococcus aureus* colonization of intravascular catheters

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Abstract

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

Methods: From the laboratory information management systems of the clinical microbiology department in 6 Dutch hospitals patients were identified who had intravascular catheters from which *S. aureus* was cultured between January 1st 2003 and December 31st 2008. Patients with demonstrated SAB between 7 days prior to catheter removal and 24 hrs after catheter removal were excluded. Clinical and demographic patient data were extracted from the patients charts. The primary risk factor was initiation of anti-staphylococcal antibiotic therapy within 24 hrs and the primary endpoint was SAB >24 hrs after IV-catheter removal. Subsequently, a systematic review and meta-analysis was performed of all observational studies evaluating the effect of antibiotic therapy for *S. aureus* IV-catheter tip colonization.

Results: 18 of 192 included patients developed subsequent SAB, which was associated with not receiving antibiotic therapy within 24 hours (OR 4.2; 95% CI: 1.1-15.6) and with documented exit site infection (OR 3.3; 95% CI: 1.2-9.3). When combining these results with those 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). In addition to the present study, 3 other studies were identified 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.

Conclusion: Early initiation of antibiotic therapy for intravascular catheters colonized with *S. aureus* prevents subsequent SAB.

Background

Catheter-associated *Staphylococcus aureus* bacteremia (SAB) is a severe healthcare-associated infection that may result in septic thrombosis, peripheral abscesses, endocarditis, and death.[2,3] Yet, catheter colonization frequently occurs without clinical signs of infection, and often without evidence of concomitant SAB. Whether such 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 indeed identified *S. aureus* colonization of intravascular 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;[1,7] 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 intravascular catheter-related infection now recommend 5 to 7 days of antibiotic treatment for patients with demonstrated *S. aureus* colonization of central vascular catheters (CVCs) but without specifying 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 both tertiary care centers. We, therefore, quantified the risks of catheter tip colonization with *S. aureus* and subsequent development of SAB, and the effects of antimicrobial therapy hereon, 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. Patients with intravascular catheters colonized with *S. aureus* during the period January 2003 – December 2008 were identified in the microbiology laboratory information management systems of these hospitals' microbiological departments. The same databases were searched for blood cultures taken between 7 days prior to intravascular catheter removal through 6 months after removal. Patients with SAB from 7 days before through 24 hours after removal of the intravascular catheter were excluded.

Patient charts were reviewed to retrieve demographic, clinical and laboratory data. The following potential risk factors were extracted: age, gender, 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, 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 of the blood culture isolate and the isolate from the IV-catheter tip. Antibiotic treatment was defined as initiation within 24 hours of (oral or intravenous) antibiotic therapy 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.[4] 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

Data was analyzed using SPSS for Windows (version 15.0). Nominal variables were analyzed by chi-square test and Fisher's exact test where appropriate, continuous variables were analyzed by the Mann-Whitney *U* test. Significance was assessed 2-sided for all variables, applying a cut-off 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). A literature search of Medline was conducted 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'. The reference lists of enrolled publications were reviewed until no further new publications were identified. The meta-analysis was carried out using Review Manager (version 5.0.24, Cochrane Collaboration, Oxford, UK). A Mantel-Haenszel analysis was performed using the fixed effects model to calculate the pooled OR and 95% CIs. I^2-statistics were used to assess heterogeneity.

Results

During the 6-year study period 450 patients with an intravascular catheters colonized with *S. aureus* were identified. 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 it could not be retrieved from their charts whether antibiotic treatment had been started within 24 hours of catheter extraction (neither patient had subsequent SAB). Finally, 192 patients were included (Table 1): 20 (10%) with tunnelled central venous catheters (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%) peripherally inserted central catheters (PICCs). The insertion site of 20 (10%) CVCs could not be retrieved. The median duration of catheter insertion was 7 days. Four cultures (2%) yielded a methicillin-resistant *Staphylococcus aureus* (MRSA).

Seventy-four patients received antibiotic therapy active against the cultured *S. aureus* within 24 hours. Thirty-seven patients were treated with specific antistaphylococcal therapy (flucloxacillin, cefazolin, clindamycin or vancomycin); another 37 patients received empiric therapy with broad-spectrum antibiotics: in 16 of these patients a switch was made to narrow-spectrum anti-staphylococcal therapy (flucloxacillin) during the course of treatment.

Eighteen patients developed a subsequent SAB at an average of 10.7 days (range 2-65 days) 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) (Table 4). 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 one 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 due to the fact that these 2 factors were inversely correlated (OR=0.5) as shown in table 2. Antibiotic therapy was more frequently prescribed in patients with fever (45% versus 19% in those without fever (OR=2.39; 95% CI 1.52-3.77)) and in patients with documented

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)	univariate analysis		multivariate analysis	
			p-value	OR (95%CI)	p-value	OR (95%CI)
Age, mean years	59	63	0.50			
Male sex	106 (61%)	9 (50%)	0.40			
Underlying conditions:						
Underlying malignancy	34 (19%)	5 (28%)	0.42			
Hematological malignancy	9 (5%)	1 (6%)	0.95			
Diabetes mellitus	30 (17%)	4 (22%)	0.62			
Hemodialysis	29 (17%)	6 (33%)	0.086	2.4 (0.86-7.1)	0.30	1.9 (0.55-6.8)
Mechanical ventilation	29 (17%)	2 (11%)	0.54			
COPD	23 (13%)	2 (11%)	0.79			
Type of catheter:						
Tunneled catheter	17 (10%)	3 (17%)	0.36			
Catheter insertion site:						
Jugular catheter	55 (32%)	6 (33%)	0.84			
Subclavian catheter	57 (33%)	5 (28%)	0.44			
Femoral catheter	24 (14%)	6 (33%)	0.060	2.7 (0.93-7.9)	0.50	1.4 (0.48-4.4)
Umbilical catheter	5 (3%)	0	1.00			
Arterial catheter	11 (6%)	1 (6%)	0.80			
PICCs	2 (1%)	0	1.00			
CVC insertion site unknw	20 (11%)	0				
Duration of catheterization, median days	7	7	0.85			

Variable	Patients without subsequent SAB (n=174)	Patients with subsequent SAB after 48h. (n=18)	univariate analysis		multivariate analysis	
			p-value	OR (95%CI)	p-value	OR (95%CI)
Use of IV catheter						
Total parental nutrition	40 (23%)	6 (33%)	0.45			
Inotropic therapy	44 (25%)	4 (22%)	0.62			
Chemotherapy	10 (6%)	1 (6%)	0.91			
Fever	47 (27%)	8 (44%)	0.12			
Documented exit-site infection	48 (28%)	9 (50%)	0.048	2.6 (0.99-7.1)	0.022	3.3 (1.2-9.3)
Immunosuppressive therapy (all)	46 (26%)	7 (39%)	0.26			
Systemic corticosteroids therapy	34 (19%)	5 (28%)	0.36			
No antibiotic therapy within 24 hours	103 (59%)	15 (83%)	0.045	3.4 (0.96-12.3)	0.034	4.2 (1.1-15.6)
Initial antibiotic therapy						
β-lactam antibiotics*	62/71 (87%)	3/3 (100%)				
Vancomycin	3 (4%)	0				
Other antibiotics†	7 (10%)	0				
Duration of antibiotic therapy, median days	9	17				
Oral therapy	6	1				

*SAB: Staphylococcus aureus bacteremia; OR: odds ratio; 95% CI: 95% confidence interval; COPD: chronic obstructive pulmonary disease; PICC: peripherally inserted central catheter; CVC: central venous catheter. *β-lactam antibiotics used: flucloxacillin 30, amoxicillin+clavulanic acid 15, cefuroxime 13, ceftriaxone 3, cefazolin 2, ceftazidime 1, cefotaxime 1. In 14 patients antibiotic therapy was switched from broad-spectrum antibiotics to flucloxacillin after one or more days. †Other antibiotics used: ciprofloxacin 3, clindamycin 2, co-trimoxazole 2. One patient received combination therapy of amoxicillin+clavulanic acid with ciprofloxacin.*

exit-site infection (39% versus 24% in those without exit-site infection; OR=1.65 (95% CI 1.07-2.54)). 3 of the 18 patients with subsequent SAB had negative blood cultures around the time of catheter extraction; in the other fifteen patients blood cultures were not drawn until they developed signs of sepsis.

We subsequently added the patient data of our previous study in which the same methods and definitions were used for 99 patients in a university center (Table 3, supplemental material).[1] In this combined cohort incidences of SAB were 4% and 16% for patients receiving (n=124) and not receiving (n=167) antibiotic therapy within 24 hours. These figures correspond to a RR 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).

The literature search identified a total of 3 retrospective cohort studies (apart from this one), for a total of 426 patients with *S. aureus* IV-catheter tip colonization.[1,6,7] All four studies used the culture-method and cut-off values described by Maki et al. (>15 colony-forming units) (4). 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 this study *S. aureus* colonization of IV-catheters was complicated by subsequent SAB (> 24 hrs after catheter removal) in 18 of 192 patients without manifest SAB at the time of catheter removal; furthermore, this complication could be prevented by prompt initiation of antibiotic therapy.

A meta-analysis of the 4 studies on this subject[1,6,7] confirmed the relation between not receiving antibiotic therapy and subsequent SAB with a pooled odds ratio 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 protocolized 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 moment the catheter was removed and the moment SAB was demonstrated, indicating that negative blood cultures do not exclude later bacteremic complications.

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

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 towards less observed protective effect of antibiotic therapy.

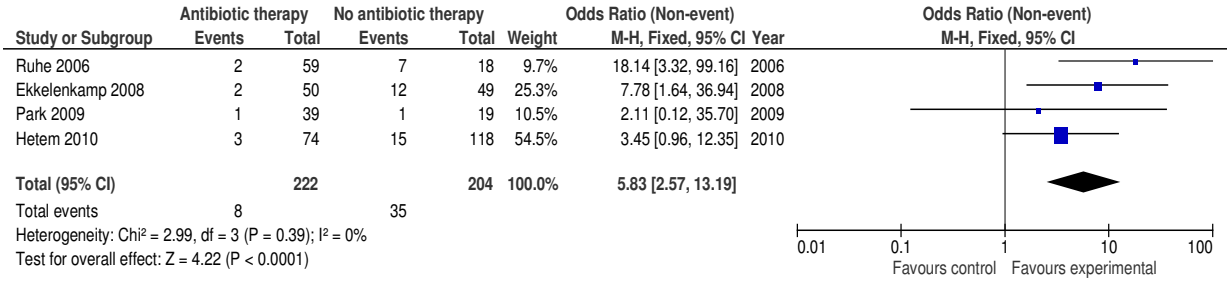
This study did not take into account whether the antibiotic therapy was specifically targeted at the *S. aureus* cultured from the IV-catheter, as long as the agent was active against the cultured isolate. In many cases broad-spectrum antibiotic therapy was started, likely because of systemic symptoms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

The IDSA-guidelines for diagnosis and treatment of IV-catheter-associated sepsis now advocate 5 to 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 recommendation is strongly supported by the findings in this study: they demonstrate its validity for patients in non-university hospitals, and almost double the number of patients it is based on. Although the IDSA recommendations would be best supported in a randomized controlled trial comparing protocolized 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 SAB and the severity of this complication make it – due to ethical considerations – practically impossible that such a trial will ever be performed. Additional comparative studies will therefore, forcibly, all be retrospective cohort studies.

Based on the results of this study and our general experience, the protocol at the hospitals where this study was performed now is 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 in hospitals where MRSA is frequent or if a patient is colonized with MRSA (alternatives may be daptomycin or linezolid), a β-lactam antibiotic (e.g. flucloxacillin, cloxacillin, cefalexin or cefazolin) in hospitals where MRSA is rare, or when a patient is colonized by methicillin-susceptible *S. aureus* (MSSA). Blood cultures should be drawn prior to 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 (e.g. cefalexin, clindamycin or linezolid). If blood cultures become positive with *S. aureus*, the patient should be treated for 14 days according to the IDSA-guidelines.

Figure 1: Meta-analysis: prophylactic antibiotic therapy for *S. aureus* colonization of intravascular catheters to prevent subsequent *SAB*



Comparison: Antibiotic therapy for patients with Staphylococcus aureus intravascular catheter colonization; outcome: Staphylococcus aureus bacteremia.

Note: In the studies by Ruhe e.a. and Park e.a. antibiotic therapy was initiated within 48 hours; in the study by Ekkelenkamp e.a. and by Hetem e.a. antibiotic therapy was initiated within 24 hours.

Table 3: Risk factors for subsequent *Staphylococcus aureus* bacteremia in patients with *S. aureus* colonization of intravascular catheters, combined analysis with data from chapter VIIa

Variable	Patients without subsequent bacteremia (n=259)	Patients with subsequent SAB after 48h. (n=32)	univariate analysis		multivariate analysis	
			p-value	OR (95%CI)	p-value	OR (95% CI)
No antibiotic therapy within 24 hours	140 (54%)	27 (84%)	0.001	4.59 (1.71 – 12.35)	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 (any)	63 (24%)	15 (47%)	0.007	2.74 (1.30 – 5.81)	0.61	1.4 (0.3 - 5.6)

OR: odds ratio; 95% CI: 95% confidence interval

References

1. 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**; 46:114-118.

2. Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, Cheng AC, Dudley T, Oddone EZ. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. **Arch Intern Med 2003**; 163:2066-2072.

3. Fowler VG Jr, Justice A, Moore C, Benjamin DK Jr, Woods CW, Campbell S, Reller LB, Corey GR, Day NP, Peacock SJ. Risk factors for hematogenous complications of intravascular catheter-associated *Staphylococcus aureus* bacteremia. **Clin Infect Dis 2005**; 40:695-703.

4. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. **N Engl J Med 1977**; 296:1305-1309.

5. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, Raad II, Rijnders BJ, Sherertz RJ, Warren DK. 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**; 49:1-45.

6. Park KH, Kim SH, Song EH, Jang EY, Lee EJ, Chong YP, Choi SH, Lee SO, Woo JH, Kim YS. Development of bacteraemia or fungaemia after removal of colonized central venous catheters in patients with negative concomitant blood cultures. **Clin Microbiol Infect 2010**; 16:742-746.

7. Ruhe JJ, Menon A. Clinical significance of isolated *Staphylococcus aureus* central venous catheter tip cultures. **Clin Microbiol Infect 2006**; 12:933-936.

VIII

Influence of the timing of therapeutic interventions on the outcome of catheter-associated *Staphylococcus aureus* bacteremia: Is there a need for speed?

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Abstract

Objectives: The objective of this study was to evaluate the effect on attributable mortality due to catheter-associated *Staphylococcus aureus* bacteremia (CA-SAB) of its two main therapeutic measures: the rapidity with which adequate antibiotic therapy is initiated and time taken to remove the intravenous catheter.

Methods: Adult patients from eight Dutch hospitals with a first episode of CA-SAB were included in this study. Inclusion was retrospective and began between July 1st 2003 and January 1st 2005, depending on the hospital; inclusion ended on December 31st 2008. Risk factors and outcome were retrieved from the patient charts. Primary risk factors were: failure to remove the intravascular catheter within 24 hours and failure to initiate antibiotic therapy within 24 hours. The primary outcome was attributable 3-month mortality. Secondary outcomes were: all cause 3-month mortality, hematogenous complications at 3 months, and all cause 12-month mortality.

Results: A total of 268 patients were included. Median age was 60 years. Co-morbidity included cardiovascular disease (44%), heart failure (15%) diabetes mellitus (23%), malignancy (28%), hematological malignancy (5.9%), and chronic obstructive pulmonary disease (9.3%). 34% of patients had tunneled catheters. The catheter was used for chemotherapy in 11% of the patients, total parenteral nutrition in 21%, and hemodialysis in 37%. Attributable 3-month mortality was 9.0%, all cause 3-month mortality 18%, and all cause 12-month mortality 30%. 27 patients (10%) suffered hematogenous complications. The catheter was removed within 24 hours in 62% of the patients, antibiotic therapy was initiated within 24 hours in 87%.

Attributable mortality was significantly associated with failure to initiate antibiotic therapy within 24 hrs and with age, but not with failure to remove the intravascular catheters within 24 hrs. The use of a tunneled intravenous catheter was associated both with a delay in catheter extraction and with a rapid initiation of antibiotic therapy. Changes in the assessment of outcome (all cause mortality, attributable mortality or hematogenous complications) determined which risk factors were found significant.

Conclusion: In this study, immediate initiation of antibiotic therapy was significantly associated with a lower incidence of attributable 3-month mortality, but not with less all cause 3-month mortality. However, studies into this subject are severely hampered by selection bias.

Introduction

Intravascular catheter-associated *Staphylococcus aureus* bacteremia (CA-SAB) is a frequent and severe complication of modern day medicine. It is associated with high rates of metastatic complications, including endocarditis, osteomyelitis, and septic thrombosis,[1] and its attributable mortality has been estimated between 8.2% and 29%.[2,3] Next to rapid initiation of antibiotic therapy, the current guidelines by the Infectious Diseases Society of America (IDSA) recommended prompt removal of the catheter in patients with CA-SAB, since this is associated with a more rapid response to treatment and fewer relapses, and because systemic antibiotics do not reach therapeutic levels inside the catheter lumen.[2,4-7] Furthermore, a previous study in 324 patients with CA-SAB reported a correlation between the time to catheter removal and the risk of hematogenous complications.[17]

In clinical practice, however, intravenous catheters and in particular tunneled catheters are sometimes deemed so valuable to a patient that attempts are made to salvage the catheter with antibiotic therapy. Small retrospective studies reported success rates of 13% up to 67%,[6,8-13] but without specifically analyzing the attributive morbidity and mortality of these salvage attempts. Insight into the risk associated with each day an intravenous catheter remains in situ in patients with CA-SAB may help guide clinicians in deciding whether or not to attempt catheter salvage.

The objective of this study was to evaluate the effect on attributable mortality of CA-SAB of its two main therapeutic measures: the rapidity with which adequate antibiotic therapy is initiated and time taken to remove the intravenous catheter (if removed at all).

Patients and methods

Data collection

Patients were retrospectively included from eight Dutch hospitals, some of which had multiple locations: the University Medical Center in Utrecht, the Diakonessenhuis in Utrecht, the St. Antonius Hospitals in Nieuwegein and Utrecht, the Amphia Hospital in Breda, the Gelre Hospitals in Apeldoorn and Zutphen, the St. Elisabeth Hospital in Tilburg, the Twee Steden Hospital in Tilburg, and the Franciscus Hospital in Roosendaal. The start of patient inclusion was based on the implementation of the latest laboratory information system in the respective hospitals and ranged from July 1st 2001 to January 1st 2003. Inclusion ended on December 31st 2008.

All patients of 18 years of age or above with SAB were retrieved from the laboratory information and management systems of these hospitals. Patients with a first episode of CA-SAB, as defined by the IDSA criteria were included in this study.[3] Patients who succumbed within on the first day of SAB or the day after were excluded. Clinical and demographical data were extracted from the patients' charts, including age, gender, underlying medical conditions, time of first positive blood culture, timing of antibiotic therapy, and 3-month mortality.

Definitions and analysis

A patient was considered to have succumbed to *S. aureus* bacteremia (attributable mortality) if a sudden clinical deterioration was documented coinciding with SAB and signs and symptoms of sepsis, which ultimately led to death and which likely reduced the life expectancy of the patient by more than a week. If relapse of SAB, endocarditis or other hematogenous complications led to death of the patient, these were also counted as attributable mortality. Day 0 was defined as the day of the first positive blood culture.

Primary risk factors analyzed were: 1. failure to remove the catheter within 24 hours, and 2. failure to start antibiotic therapy within 24 hours. All gathered relevant patient characteristics were analyzed as secondary risk factor. The primary outcome was the 3-

month mortality, attributable to CA-SAB. Secondary outcome measures were the all cause 3-month mortality, the 3-month hematogenous complications of CA-SAB, and the all cause 12-month mortality.

Statistical analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Baseline statistics for different subgroups were analyzed using two-tailed Pearson's χ^2, Fisher's exact test or Mann Whitney U test where appropriate. Odds ratio's and p-values were calculated. Variables with a p-value <0.1 were analyzed by multivariate logistic regression (backward stepwise method).

Literature search

A search for articles reporting on catheter salvage outcome was performed in PubMed with the terms "*Staphylococcus aureus*", "intravascular catheter", and "salvage". A search for articles reporting on outcome of CA-SAB was performed in PubMed with the terms "*Staphylococcus aureus*", "bacteremia" and "intravascular catheter".

A search for articles reporting on outcome of SAB was performed in PubMed with the terms "*Staphylococcus aureus*" and "bacteremia; this search was not intended to be comprehensive and articles were screened by title.

Results

Patient demographics

A total of 268 patients with CA-SAB complied with the inclusion criteria (Table 1). The median age was 60 years (interquartile range 46-71 years), 144 patients (54%) were male. Co-morbidity included, amongst others, cardiovascular disease in general (44%), heart failure (15%), chronic obstructive pulmonary disease (9.3%), diabetes mellitus (23%), malignancy (28%), and hematological malignancy (5.9%). One hundred and seven patients had tunneled catheters (34%). The catheter was used for chemotherapy in 11% of the patients, total parenteral nutrition (TPN) in 21%, and hemodialysis in 37%. Attributable 3-month mortality was 9% (Table 2), all cause 3-month mortality was 18%, and all cause 12-month mortality was 30%. One patient (0.4%) had CA-SAB caused by methicillin-resistant *S. aureus* (MRSA).

Intravascular catheter-removal

One hundred and nine catheters (41%) were removed on day 0, 56 (21%) the next day, 34 (13%) two days later, 38 (14%) between 3 and 14 days later, and 25 (9.3%) more than 14 days later or not at all. Six patients (2.2%) died with their intravenous catheters in situ (Figure 1). Patient characteristics that were significantly associated with failure to rapidly remove the catheter (after >24 hours) were malignancy, hematological malignancy, immunosuppressive therapy, hemodialysis, chemotherapy, and the presence of a tunneled catheter; receiving TPN was associated with removal within 24 hours. In multivariate analysis, the patient characteristics which remained significantly associated with delayed catheter removal were malignancy, the use of a tunneled catheter and hemodialysis; receiving TPN remained significantly associated with rapid catheter removal.

Antibiotic therapy

Antibiotic therapy was started before day 0 in 7 patients (2.6%), on day 0 in 136 patients (51%), on day 1 in 72 (27%), on day 2 in 20 patients (7.5%), on day 3 in 5 patients (1.9%),

Table 1: patient characteristics

Patient characteristics	N = 268	Catheter removed within 24 hrs (N=165)	P-value[1]	AB therapy within 24 hrs (N=215/248)	P-value[1]
Sex (male)	144 (54%)	95 (58%)	0.098	115 (53%)	0.66
Age, median (IQR)	60 (46-71)	60 (46-71)	0.61	60 (46-72)	0.54
Co-morbidity / risk factors					
Cardiovascular disease	119 (44%)	76 (46%)	0.49	94 (44%)	0.40
Heart failure	39 (15%)	24 (15%)	1.0	31 (14%)	0.60
COPD	25 (9.3%)	15 (9.1%)	0.87	22 (10%)	0.45
Malignancy (any)	75 (28%)	37 (22%)	**0.010 OR=0.49 (0.29-0.85)[3]**	61 (28%)	0.62
Hematological malignancy	16 (5.9%)	6 (36%)	**0.041 OR=0.35 (0.12-1.0)**	14 (7 %)	1.0
Diabetes mellitus	63 (23%)	37 (22%)	0.60	56 (26%)	0.08[4]
Corticosteroid use	27 (10%)	13 (7.8%)	0.13	21 (10%)	0.75
Immunosuppressive therapy (any)	71 (27%)	35 (21%)	**0.013 OR=0.50 (0.29-0.87) [3]**	55 (26%)	0.87
Tunneled catheter	92 (34%)	25 (15%)	**<0.001 OR=0.096 (0.05-0.17) [3]**	80 (37%)	**0.032 OR=2.7 (1.1-6.7)[4]**
Catheter use					
Total parenteral nutrition	55 (21%)	43 (26%)	**0.004 OR=2.7 (1.3-5.4) [3]**	43 (20%)	0.81
Chemotherapy	29 (11%)	9 (5.4%)	**<0.001 OR=0.24 (0.10-0.55) [3]**	24 (11%)	0.15
Hemodialysis	99 (37%)	54 (32%)	**0.024 OR=0.57 (0.35-0.93) [3]**	87 (40%)	**0.034 OR=2.5 (1.05-6.1)[4]**

Table 1: patient characteristics

Patient characteristics	N = 268	Catheter removed within 24 hrs (N=165)	P-value[1]	AB therapy within 24 hrs (N=215/248)	P-value[1]
Therapy					
Antibiotics initiated within 24 hours	215/248 (87%)	132/154 (86%)	0.56		
Catheter removed within 24 hours	165 (62%)			132 (61%)	0.56
Empiric therapy with beta-lactam	215 (80%)				
Outcome					
3-month mortality, all cause	49 (18%)				
3-month mortality, attr to CA-SAB	24 (9.0%)				
3-month complications[2]	27 (10%)				
12-month mortality, all cause	80 (30%)				
12-month complications[2]	31 (12%)				

OR: odds ratio; COPD: chronic obstructive pulmonary disease; CVC: central venous catheter; CA-SAB: catheter-associated Staphylococcus aureus bacteremia; [1]for risk factors with a statistically significant association ($p<0.05$) the odds ratios are provided with the 95% confidence intervals between parentheses; [2]endocarditis, abscess, septic thrombosis or osteomyelitis; [3]in multivariate analysis the following patient characteristics remained significantly associated with catheter removal within 24 hours: tunneled catheter, malignancy (any), total parenteral nutrition and hemodialysis; [4]in multivariate analysis the following patient characteristics remained significantly associated with initiation of antibiotic therapy within 24 hours: tunneled catheter and hemodialysis.

and on day 3 or later in 5 patients (1.9%); three patients (1.1%) did not receive antibiotic therapy, two of which died (Figure 2). For 20 patients (7.5%) the exact day antibiotic therapy was started could not be retrieved. The low percentage of MRSA bacteremias made that all empirical antibiotic therapies could be considered *adequate*.

Early initiation of antibiotic therapy was significantly associated with the use of a tunneled catheter and with hemodialysis (Table 1). No relation was found between early initiation of antibiotic therapy and early catheter removal.

Analysis of 3-month mortality, 3-month attributable mortality, and 3-month complications

The relation between catheter removal / initiation of antibiotic therapy is most accurately depicted in figures 1 and 2. Such a graphic representation, however, does not lend itself to easy interpretation. For statistical analysis, a cut-off point was chosen between day 1 and day 2, i.e. at approximately 24 hours after the first positive blood cultures were drawn.

Table 3A shows the results of univariate analysis of the relation between patient characteristics and therapeutic interventions, and the outcome measures. As can be appreciated from the table, the risk factors varied for the different outcomes. In univariate analysis risk factors associated with attributable mortality were: age, heart failure and failure to initiate antibiotic therapy within 24 hours. In multivariate analysis, age and failure to initiate antibiotic therapy remained as significant risk factors (Table 3B). Risk factors significantly associated with all cause 3-month mortality were: age, cardiovascular disease, heart failure, and immunosuppressive therapy. In multivariate analysis the following risk factors remained significant: age, heart failure, and immunosuppressive therapy. No significant risk factors for hematogenous complications of CA-SAB were identified. Risk factors for all cause 12-month mortality were heart failure, malignancy, chemotherapy, and immunosuppressive therapy.

No significant relation was found between early catheter removal and any of the outcome measures.

Risk factors for mortality in patients with catheter in situ after 48 hours

It may be argued that patients with CA-SAB whose IV-catheter have not been removed after 48 hours (but who did receive antibiotic therapy) should be considered "salvage attempts". Sixty-eight patients had their catheters in situ past day two and could, therefore, be considered salvage attempts. Ten (15%) died, three of which were considered attributable deaths (Figure 1). Risk factors for death and attributable death were analyzed in this population, to determine whether some patients would especially at risk for death if their catheters are not removed. However, the only significant association found was between age and total 3-month mortality (p=0.025 when tested by Mann Whitney U test).

Discussion

The objective of this study was to quantify the influence of speed of the two main therapeutic interventions on the outcome of CA-SAB. We found a significant association between early initiation of antibiotic therapy and attributable mortality, but not between early removal of intravascular catheters and attributable mortality. The other risk factor significantly associated with attributable mortality was age. The use of a tunneled intravenous catheter was associated both with a delay in catheter extraction and with a rapid initiation of antibiotic therapy. Changing the outcome measure (to all cause 3-month mortality, all cause 12-month mortality or hematogenous complications) also changed the significant risk factors.

It is important to realize the limitations of this study. In the first place, it was a retrospective study, compromising the accuracy and completeness of patient data. Some parameters could not be determined at all, such as the actual day a patient experienced his first symptoms of sepsis. Patient delay (for out-patients) and diagnostic delay could not be corrected for, nor could a reliable subdivision in different grades of sepsis be made or could APACHE scores be determined. Secondly, as is the case in all studies evaluating the effect

Table 2: Patients with mortality attributable to catheter-associated *Staphylococcus aureus* bacteremia

Nr	SAB to death[1]	Description
1	0	Admitted with progressive heart failure. Relatively stable condition until development of SAB.
2	0	Admitted with atrial fibrillation and heart failure. Rapid deterioration and death due to sepsis.
3	0	Antiphospholipid syndrome and hemodialysis-dependence since one week. CA-SAB and thrombosis of the basilar artery.
4	1	Chronic myeloid leukemia, non-myeloablative stem cell transplant. Graft versus host disease. Succumbed to CA-SAB from port-a-cath.
5	1	Renal transplant and chronic pancreatitis. Admitted for adhesion ileus. After operative procedure sudden deterioration and death due to sepsis.
6	2	Dotter procedure of the mitral valve, complicated by mitral insufficiency. Mitral valve reconstruction. Initial recovery, but sudden deterioration and death due to sepsis.
7	2	Sub-arachnoidal hemorrhage. Initial recovery with CSF-punctures. Developed CA-SAB, sepsis and infarction of medial cerebral artery, with rapid death.
8	2	Patient with a Grawitz tumor, prostate cancer and renal insufficiency. Admitted for construction of Cimino-shunt. One week after hospitalization drop in blood pressure not responsive to fluid administration. Blood cultures positive for *S. aureus* and enterococci. Patient became comatose and succumbed.
9	2	Chronic hepatitis C infection and pre-existing heart disease. Admitted with renal insufficiency and liver cirrhosis. Sudden deterioration and death due to sepsis.
10	3	Chronic renal insufficiency, prior heart disease. Admitted with anuria, cardiac arrhythmia and angina pectoris. Developed CA-SAB and succumbed within 24 hours. No therapy initiated due to severe underlying disease.
11	3	Patient with history of dilating cardiomyopathy, heart failure, gout and diabetes mellitus. Admitted because of cardiac arrhythmia's. Progressive heart failure during hospitalization with need for inotropy. Development of CA-SAB, and rapid death.
12	3	Patient with severe COPD. Admitted for heart failure and kidney failure. Developed CA-SAB and multi-organ failure on sixth day of hospitalization and succumbed.
13	4	RA with many complications, and MGUS treated with chemotherapy. Sepsis during neutropenic stage, for which the therapy was halted after 48 hours.
14	5	Severe psychiatric morbidity. Admitted with pneumonia and respiratory insufficiency. Initial improvement on antibiotic treatment. Detubation. Developed CA-SAB and (again) respiratory insufficiency. Patient declined all therapy and succumbed.
15	5	Patient with hypertension, aneurysm of the abdominal aorta, and atrial fibrillation. Admitted because of kidney failure. Patient developed CA-SAB a week after initiation of hemodialysis (started at week 6 of

		hospitalization), and succumbed.
16	6	Patient admitted for bleeding of the upper gastro-intestinal tract. Developed CA-SAB with renal failure, ARDS and hypotension, and succumbed.
17	6	Patient with heart failure. Developed CA-SAB two weeks after hospitalization and succumbed.
18	8	Admitted for trauma with multiple fractures and subdural hematoma. Developed CA-SAB and ARDS one month after hospitalization. Succumbed.
19	11	Sjögren's syndrome, Raynaud's disease and hemodialysis. Admitted with CA-SAB with S. aureus and enterococcus. Developed endocarditis and succumbed to cerebral septic emboli.
20	11	Congenital heart disease, for which many operative procedures. Admitted with heart failure. Succumbed to combination of CA-SAB and bleeding of the upper GI-tract.
21	12	Hemodialysis patient, admitted with CA-SAB. Died due to sepsis with persisting SAB.
22	17	Patient with stomach cancer. Admitted with gastric retention. Developed CA-SAB, complicated by endocarditis to which the patient succumbed.
23	28	Breast cancer; chemotherapy administered over port-a-cath. Admitted with CA-SAB. Progressive deterioration with thrombosis of the portal vein and rifampicin-induced hepatitis.
24	32	Hemodialysis patients. Admitted with CA-SAB. Developed endocarditis and succumbed to hemodynamic instability.
25	33	Breast cancer; treatment with chemotherapy. Admitted with CA-SAB and developed respiratory insufficiency. Conclusion of obduction: death due to sepsis.
26	36	Patient with severe asthma and high dose corticosteroid use. Admitted for exacerbation of asthmatic brochitis. Hospitalization complicated by CA-SAB, which was treated for two weeks. Three weeks after end of treatment the patient was readmitted with SAB of unknown focus and succumbed within 24 hours.
27	39	Patient with kidney transplant and prior heart disease. Admitted due to renal transplant rejection. Developed CA-SAB and bacteremia with *Listeria monocytogenes*. Persisting bacteremia and death.
28	40	Admitted with bowel perforation. Developed CA-SAB postoperatively. Catheter exchanged. Patient discharged with Hickman-catheter for TPN. Re-admitted within 48 hours with SAB, lung abscesses and respiratory insufficiency. Succumbed.
29	43	Patient hospitalized for almost a year and operated several times, due to necrotizing pancreatitis. Developed CA-SAB. The catheter was exchanged but without a catheter-free interval. One month later second episode of CA-SAB with clinical deterioration and death on day 14 of the second episode.
30	58	Hemodialysis patient, admitted with CA-SAB. Developed endocarditis. Mitral valve replacement with bioprosthesis. Developed mediastinitis and sepsis postoperatively, and succumbed.

[1]days between occurrence of SAB and death; CA-SAB: catheter-associated Staphylococcus aureus bacteremia; SAB: S. aureus bacteremia; RA: rheumatoid arthritis; MGUS: ; ARDS: acute respiratory distress syndrome ; TPN: total parenteral nutrition.

Table 3A: Risk factors for adverse outcome in patients with CA-SAB

Risk factors	All patients N = 268	3-month mortality N=49	p-value[1]	3-month attr mortality; N=24
Sex (male)	144 (54%)	27	0.88	16
Age, median (IQR)	60 (46-71)	66 (55-75)	**0.027; OR[3]=1.022 (1.001-1.043)**	70 (57-75)
Cardiovascular disease (any)	119 (44%)	28	**0.047; OR=1.9 (1.0-3.5)**	13
Heart failure	39 (15%)	15	**<0.001; OR=3.6 (1.7-7.5)**	8
COPD	25 (9.3%)	4	1.0	2
Malignancy (any)	75 (28%)	16	0.42	4
Hematological malignancy	16 (5.9%)	6	0.086; OR=2.9 (1.0-8.5)	0
Diabetes mellitus	63 (23%)	14	0.36	6
Corticosteroid use	27 (10%)	8	0.11	2
Immunosuppressive therapy (any)	71 (27%)	20	**0.012; OR=2.3 (1.2-4.4)**	7
Tunneled catheter	92 (34%)	13	0.20	7
Total parenteral nutrition	55 (21%)	9	0.68	3
Chemotherapy	29 (11%)	8	0.17	3
Hemodialysis	99 (37%)	14	0.18	6
Antibiotics initiated within 24 hrs	215/248 (87%)	38	0.21	16
Catheter removed within 24 hrs	165 (62%)	34	0.21	18
Empiric R/ beta-lactam	215 (80%)	39	0.48	18

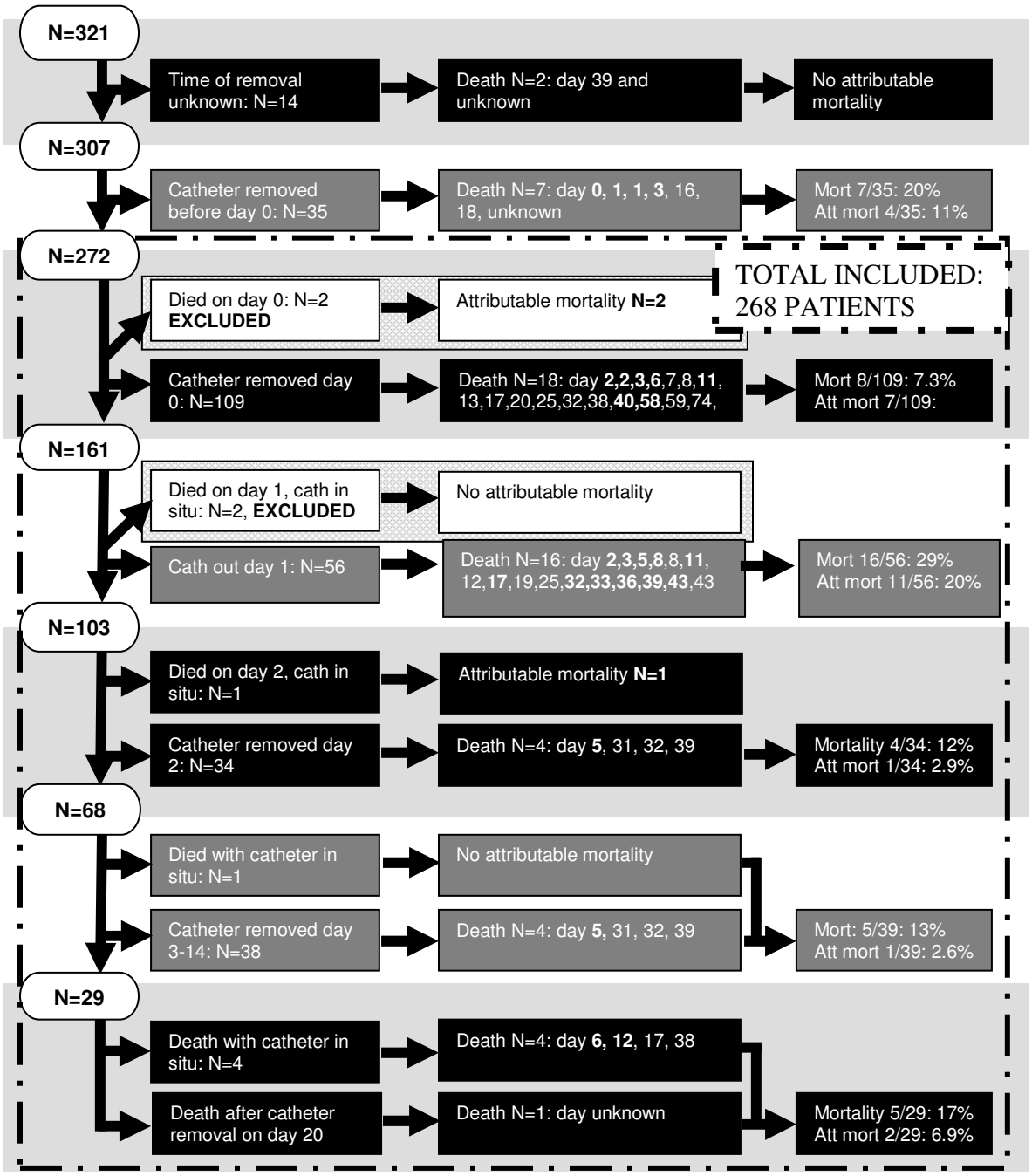
IQR= interquartile range; OR=odds ratio; COPD=chronic obstructive pulmonary disease; [1]for risk factors with a p-value <0.1 the odds ratios are provided with the 95% confidence intervals

Table 3A: Risk factors for adverse outcome in patients with CA-SAB

p-value[1]	3-month complications N=27	p-value[1]	12-month mortality N=80	p-value[1]
0.19	15	0.86	40	0.40
0.021; OR[3]=1.035 (1.004-1.067[3])	62 (47-68)	0.96	62 (47-68)	0.082
0.31	9	0.22	41	0.14
0.006; OR=3.4 (1.4-8.7)	3	0.78	21	**<0.001; OR=3.4 (1.7-6.7)**
1.0	2	1.0	9	0.48
0.20	8	0.84	30	**0.024; OR=1.9 (1.1-3.3)**
0.38	1	1.0	9	**0.024; OR 3.3 (1.2-9.1)**
0.86	9	0.20	21	0.49
1.0	3	0.74	12	0.081; OR 2.0 (0.91-4.6)
0.76	6	0.60	32	**0.001; OR=2.5 (1.4-4.5)**
0.58	10	0.76	29	0.67
0.43	8	0.22	12	0.14
0.73	3	1.0	15	**0.006; OR=2.9 (1.3-6.2)**
0.20	8	0.41	29	0.88
0.022; OR=0.31 (0.12-0.81)	22	0.38	67	0.85
0.16	20	0.16	45	0.24
0.26	27	0.13	61	0.10

between parentheses; [2]11 patients died within 48 hours, of which 9 deaths were attributable to CA-SAB; [3]odds per year of age; [4]odds ratio not determined due to count of 0 in one cell.

Figure 1: Timing of catheter removal and mortality in CA-SAB



of delay in catheter removal and delay in initiation of antibiotic therapy, this study was observational. The relation between intervention and outcome was heavily biased by the patients' clinical presentation and their co-morbidity. Physicians may decide to delay start of antibiotic therapy and catheter removal because a patient suffers limited symptoms or has a clinical picture which at first is not unequivocal; on the other hand, this decision may result from an approach in which interventions are minimized in a terminal patient. Catheter salvage may also be inspired by the patients' dependence on a CVC to facilitate vascular access for hemodialysis, chemotherapy or total parenteral nutrition. Only a randomized clinical trial could reliably estimate the contribution of rapid catheter removal and antibiotic initiation on the outcome of CA-SAB, but such a study would face all sorts of ethical impossibilities: it would be unacceptable to leave an infected intravascular catheter in situ when possible to remove, or to postpone the initiation of antimicrobial therapy in patients suspected of CA-SAB.

Analysis and interpretation of data on outcome of CA-SAB is difficult, due to the high co-morbidity of patients who experience this complication. In our cohort, we found a 18% 3-month mortality, but only a 9.0% attributable mortality. This is grossly in concordance with most studies reporting on mortality in SAB (Table 4A), be it that the mortality was relatively low. Attributable mortality as a fraction of total mortality in SAB has been reported between 40% and 80%. The maximum effect expected from any intervention on total mortality (in other words: if absolutely nobody dies from *S. aureus* infection) would therefore be a reduction of 40-80%. SAB, however, is often rapidly fatal, and in many studies patients who succumb within 48 hours are deemed "impossible to save" and excluded from analysis. In this study a third of the attributable mortality occurred within 48 hours; excluding this group of patients lowers the theoretical maximum reduction in mortality to 25-55%. Furthermore, the 40-80% attributable mortality is in all likelihood an overestimation of the measurable effect on total mortality. In our judgement, a relevant percentage of the patients whose death was attributed to CA-SAB, had a life expectancy of less than three months; in some the occurrence of sepsis even led to the decision to halt most

or all therapeutic interventions. The relatively limited contribution of CA-SAB to the mortality in this patient group renders achieving enough statistical power to demonstrate effect of therapy on survival highly problematic.

Rapid initiation of antibiotic therapy

Nonetheless, in the present study a significant association was found between rapid initiation of antibiotic therapy and a favorable outcome. The odds ratio of 0.31 suggests a possible 70% reduction of the attributable mortality when antibiotics are initiated within 24 hours, but without a significant effect on the total 3-month mortality. No previous studies evaluated the effect of rapid antibiotic therapy on mortality in CA-SAB. One previous study reported that it did not find a significant association between time to start of effective antibiotic therapy and hematogenous complications (Table 4A).[14]

The results of previous studies on SAB (all causes) are not unequivocal regarding the effect of rapid antibiotic therapy: some demonstrated an effect, while others did not (Table 4B). This study differs from previous ones in two essential elements. First, the cut-off point for timely initiation of therapy was set at 24 hours instead of 48 hours. A cut-off point of 48 hours may offer practical advantages when performing a study, but in a sometimes rapidly fatal disease such as *S. aureus* sepsis it may well be too long. In the second place, thanks to the virtual absence of MRSA, empiric antibiotic therapy was always *adequate* in the present study, whereas in others a large proportion of the patients received initial therapy with antibiotics to which the infecting strain was tested resistant. Although far from optimal, antibiotic therapy to which a micro-organism is classified as resistant, or which is considered suboptimal (e.g. aminoglycoside monotherapy), is often still more active than no antibiotics at all. Having to take such patients into account severely complicates the analysis of the relation between antibiotic therapy and outcome, thus requiring even larger cohorts to achieve the necessary statistical power to demonstrate such a relation.

Rapid removal of the intravascular catheter

Previous studies have consistently demonstrated that failure to remove an intravascular catheter is associated with therapeutic failure, persistent bacteremia, recurrence of bacteremia, and hematogenous complications.[6,12,14] A higher mortality compared to control groups has, thus far, not been demonstrated, nor has it in the present study. This may either be because the effect is too small to be measurable, or because the decision to remove the catheter is heavily biased by the patients' conditions. For instance, we found that the time which elapsed between positive blood culture and catheter removal was significantly associated with hemodialysis, underlying malignancy and the presence of a tunneled catheter.

IDSA-guidelines recommend prompt removal of intravascular catheters in case of CA-SAB, but salvage is still often attempted, especially when infected IV-catheters are tunneled or completely implanted. A literature search we performed identified seven studies that reported on catheter salvage attempts in CA-SAB patients (Table 4C). The results of these studies is generally similar: in all studies 30-70% of the salvage attempts are successful, but also in all studies a number of patients succumb to *S. aureus* sepsis after several days of antibiotic therapy (Table 4C). It would be expected that the group in which salvage is attempted, were clinically stable with their infection suppressed by antibiotic therapy, but from these studies it has to be concluded that this is often not the case. Inherently, these patients died without receiving maximal treatment. Also in the present study two patients succumbed to CA-SAB with IV-catheters in situ past day 3. (On day 6 and day 12 respectively). It appears that selection of patients in which salvage can be safely attempted is not feasible. We therefore believe that the risk to which patients are exposed overshadows the perceived potential benefits of catheter salvage in CA-SAB in the majority of patients.

Table 3B: Risk factors associated with 3-month mortality (multivariate regression)

Risk factor	3-month mortality		Attributable 3-month mortality	
	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
Age	p=0.007;	**p=0.041; OR[1]=1.023 (95% CI: 1.001-1.046)**	p=0.026; OR=1.038[1]	**p=0.022; OR=1.034[1] (95% CI: 1.002-1.068)**
Cardiovascular disease	p=0.047;	NS	NS	-
Heart failure	p<0.001;	**p=0.001; OR=3.8 (95% CI: 1.7-8.4)**	p=0.011;	NS
Hematological malignancy	p=0.086;	NS	NS	**-**
Immunosuppressive therapy	p=0.012;	**p=0.001; OR=3.2 (95% CI: 1.6-6.6)**	NS	**-**
Antibiotic therapy within 24 hours	NS	**-**	p=0.006;	**p=0.026; OR=0.31 (95%-CI: 0.12-0.83)**

OR= odds ratio; n.s.: not significant; 95% CI: 95% confidence interval; NS=not significant; [1]Increase in odds per year of age

Table 4A: Studies reporting on mortality and complications in catheter-

Study	Included patients	Mortality 3 months	Mortality 12 months	Attributable mortality
Lesens, prosp[15]	60 adults with CA-SAB (2001-2002)	N.R.	N.R.	10 (17%)
Zeylemaker, retrosp[3]	49 adult patients (1994 -96) with CA-SAB	N.R.	19 (39%)	14 (29%)
Fowler, prosp[14]	324 adult patients with CA-SAB (1994-2001), exclusion of patients who died before week 12.	N.R.	N.R.	N.R.
Present study	268 adult patients with CA-SAB	49 (18%)	80 (29%)	24 (9.0%)

Conclusion

Immediate initiation of antibiotic therapy appears potentially life-saving in patients with CA-SAB. A reduction of attributable mortality by rapid catheter removal has, thus far, not been established, but this may well be due to severe methodological difficulties in addressing the issue. However, salvage attempts are associated with considerable numbers of septic deaths, and therefore these attempts should be reserved for cases where no alternatives exist.

associated *Staphylococcus aureus* bacteremia, published since 2000

Complications	Effect of rapid AB initiation	Effect of rapid catheter removal	Primary outcome
N.R.	N.R.	N.R.	Attr mort
24 (49%), including attr mort, at 1 year	N.R.	N.R.	Mort / complications
42 (13%), at 12 weeks	No signif assoc w/ development of complications	Signif relation of complications w/ failure to (rapidly) remove catheter	Endocard, vertebral osteomyelitis or arthritis
27 (10%) at 3 months; 31 (12%) at 12 months	See Table 3	No effect demonstrated on (attr) mort	Attr mort

Table 4B: Cohort studies reporting mortality/complications

Study	Included patients[a]	Exclusion based on early death?	In-hospital mortality	Mortality 3 months
Soriano, prosp[16]	908 pat w/ SAB (91-98)	No	N.R.	N.R.
Jensen, retrosp[17]	278 patients w/ SAB (94-96)	Death w/i 3 days excl	N.R.	95 (34%)
Fowler, prosp[18]	724 adults w/ SAB (94-99), includes pat from previous study	Death prior to return of blood culture excl	N.R.	157 (22%)
Diekema, prosp[19]	184 pat > 16 y w/ SAB (99-00)	No	43 (23%)	N.R.
Melzer, prosp[20]	815 pat > 16 y w/ SAB (95-00)	No	172 (21%)	N.R.
Lodise, retrosp[21]	167 pat w/ hospital-acquired SAB (99-01)	Death within 48 hours excluded	53 (32%)	N.R.
Lesens, prosp[15]	166 adults w/ SAB (01-02)	No	N.R.	63 (38%)
Fätkenheuer, retrosp[22]	229 pat w/ SAB (97-00)	No	52 (23%)	N.R.
Wertheim, prosp[23]	81 pat w/ hospital-acquired SAB (99-01),	No	26 (32%)	N.R.
Kaech, retrosp[24]	308 adults with SAB (1998-2002)	No	59 (19%)	N.R.
Kim, retrosp[25]	238 pat > 15 y w/ SAB (98-01); includes results previous publication	No	N.R.	103 (43%)
Wang, retrosp[26]	1148 pat w/ SAB (90-04)	No	506 (44%) at 30 days	N.R.
Rieg, retrosp + prosp[27]	521 pat >16 years w/ SAB (02-07)	No	113 (22%)	N.R.
Ammerlaan, retrosp[28]	334 adults w/ SAB (2007)	Death within 1 day excluded	80 (24%) at 30 days	N.R.
Price, prosp[29]	100 pat w/ SAB (06-07)	No	38 (38%) at 30 days	N.R.

[a]years of patient inclusion between brackets; AB: antibiotic; attr: attributable; endocard: endocarditis; mort: mortality; assoc: associated; infect: infection; metast: metastatic; NR: not reported; pat: patients; prosp: prospective; retrosp: retrospective; sign: significant; w/: with; w/i: within

in SAB (since 2000, list not comprehensive)

Mortality 12 months	Attr mort	Complications	Effect rapid initiation of AB therapy	Effect of adequate antibiotic therapy
N.R.	110 (12%)	65 (7%) septic metastases	N.R.	Sign assoc attr mort and inadequate empirical therapy
N.R.	N.R.	45 (16%) = secondary foci	N.R.	N.R.
N.R.	86 (12%)	310 (43%) = secondary foci	N.R.	No significant association with outcome
N.R.	N.R.	N.R.	N.R.	N.R.
N.R.	67 (8%)	54 (7%) = disseminated infect.	N.R.	No significant association with attr mortality
N.R.	39 (23%)	N.R.	Delay > 44.75 hrs assoc w/ attr mort	AB therapy only counted when adequate
N.R.	33 (20%)	37 (22%) = metastatic infect. or endocarditis	N.R.	N.R.
82 (36%)	N.R.	N.R.	Delay >48 hrs not associated w/ outcome	AB therapy only counted when adequate
N.R.	16 (20%)	N.R.	N.R.	N.R.
N.R.	N.R.	73 (24%) = sec foci	No significant association w/ outcome	No significant association with outcome
N.R.	79 (33%)	N.R.	N.R..	Signif assoc inad therapy w/ all cause mort; not w/ attr mort
N.R.	N.R.	N.R.	N.R.	N.R.
N.R.	N.R.	189 (36%) = metast infect; 56 (11%) endocard	N.R.	N.R.
N.R.	N.R.	N.R.	N.R.	No significant association with outcome
N.R.	N.R.	27 (27%) = secondary focus or relapse	Delay > 48 hrs assoc. with complications	AB therapy only counted when adequate

Table 4C: Studies reporting on catheter-salvage attempts in

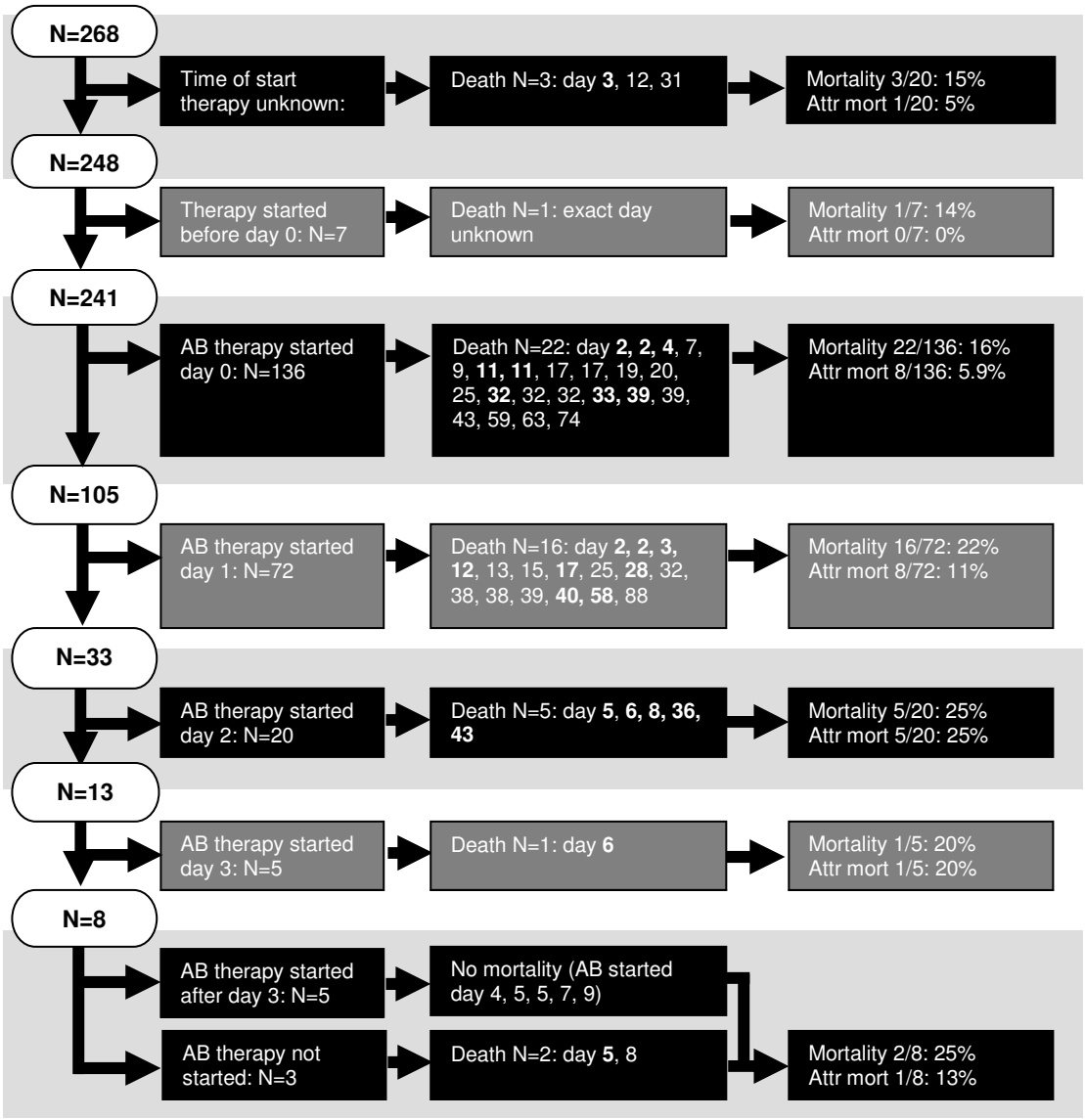
Study	Number of patients with CA-SAB	Remarks	Total mortality in salvage attempts
Dugdale, retrosp[6]	37 pat w/ Hickman and CA-SAB	18 removed initially	3/19 (16%)
Saad, prosp[30]	19 hemodialysis pat w/ CA-SAB	2 patients with endocarditis (unclear if catheter salvage was attempted)	0
Rubin, retrosp[31]	6 pediatric hematology patients w/ implantable venous ports	Study period 1991-1993, published 1999	0
Saxena, prosp[11]	36 episodes in of SAB in hemodialysis pat.	Protocol dictated salvage attempt	13/36 episodes
Kim[a], retrosp[32]	32 neutropenic cancer pat w/ Hickman in situ after 3 days (appropr empiric AB therapy) or 6 days (inappr AB therapy).	8/32 patients excluded: 3 patients due to early death, 5 patients due to catheter extraction before day 3-6	13/32 episodes (40%), 13/29 patients (45%).
Pigrau[b], retrosp[33]	87 patients w/ CA-SAB. Excl: death before end of treatment (14 days iv antibiotics + antibiotic lock)	20 salv attempts described. 12 patients died of sepsis before day 8: excl w/o specification of cath salv att	Unclear, deaths up to day 8 were excluded
Fernandez-Hidalgo[b], retrosp + prosp[9]	20 pat w/ long term CVC. Excl: Pocket-, tunnel or exit-site infection; complicated CA-SAB, hemodynamic instability.	Retrospective inclusion; in prospective study only salvage attempts for CA-SAB if no alternative venous access.	N.R.
Mokrzycki, prosp[12]	71 hemodialysis pat with tunneled cuffed cath	15 salvage attempts (+ 7 exchanges over guidewire). 8/15 treatment failure (=septic death or recurrence SAB)	N.R.
Maya, prosp[34]	113 hemodialysis pat, treated with antibiotic lock therapy	Protocol dictated salvage attempt	24/113 (21%, at 6 months)
Park[a], retrosp[35]	48 hematological pat w/ Hickman cath. Excl: cath removal w/i 72 hrs	Protocol dictated salvage attempt	7/48

AB: antibiotic; att: attempt; CA-SAB: Catheter-associated Staphylococcus aureus bacteremia; cath: catheter; N.R.: not reported; w/: with; w/i: within; w/o: without; [a]same study-group; [b]same study-group

patients with catheter-associated *Staphylococcus aureus* bacteremia

Mort attr to *S. aureus* in salv att	Successful salv of total	Successful salv of attempts	Attr deaths / salv catheter	Conclusions of study
3	8/37 (18%)	8-12 / 19 (4 patients follow-up < 6 wks)	3 deaths / 8-12 salv cath	In (most) patients w/ Hickman catheters who have SAB, early cath removal should be considered.
0	8 / 19 (42%)	8/12 (67%)	0	No specific recommendation on CA-SAB.
0	4/6 (67%)	4/6 (67%)	0	A trial on port salvage can be considered in CA-SAB.
N.R.	Not reported per individual micro-organism	Not reported per individual micro-organism	N.R.	Salv att may be recommended before actually sacrificing the vascular access in CR-BSI.
In 7 cases mortality attributable to *S.aureus*	12/32 (37%)	12/24 (37%)	7 deaths / 12 salv cath	Catheter salvage can be attempted in *S. aureus* bacteremia among neutropenic cancer patients.
Unclear, deaths up to day 8 were excluded	11/87 (13%)	Unclear, deaths up to day 8 were excluded	1-13 deaths? / 11 salv cath	Recommends catheter removal whenever possible, always when clinical situation deteriorates or if implantation is recent.
1 patient with septic shock and death (on day 7)	N.R.	11/20 (55%)	1 death / 11 salv cath	Conservative management of CA-SAB with AB lock and systemic therapy should be avoided, but may be option in selected patients.
5/71 total patient group death due to sepsis	7/71 (10%)	7/15 (47%)	0-5 deaths? / 7 salv cath	Failure to remove or exchange a tunneled cuffed cath in SAB assoc w/ 8-fold higher risk of treatment failure.
N.R.; 11 serious complications	15/113 (13%)	15/113 (13%)	N.R.	Routine AB lock therapy not appropriate for hemodialysis pat w/ CA-SAB.
2	29/56 (52%)	29/43 (60%)	2 deaths / 29 salv cath	Emphasis on salvage success.

Figure 2: Timing of antibiotic therapy and mortality in CA-SAB



Bold type indicates attributable mortality

References

1. Raad I, Hanna H, Maki D. Intravascular catheter-related infections: Advances in diagnosis, prevention, and management. **Lancet Infect Dis. 2007**; 7:645-657.

2. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, 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**; 49:1-45.

3. Zeylemaker MM, Jaspers CA, van Kraaij MG, Visser MR, Hoepelman IM. Long-term infectious complications and their relation to treatment duration in catheter-related *Staphylococcus aureus* bacteremia. **Eur J Clin Microbiol Infect Dis. 2001**; 20:380-384

4. Wolf HH, Leithauser M, Maschmeyer G, Salwender H, Klein U, Chaberny I, et al. Central venous catheter-related infections in hematology and oncology : Guidelines of the infectious diseases working party (AGIHO) of the German society of hematology and oncology (DGHO). **Ann Hematol. 2008**; 87:863-876.

5. Fatkenheuer G, Buchheidt D, Cornely OA, Fuhr HG, Karthaus M, Kisro J, et al. Central venous catheter (CVC)-related infections in neutropenic patients--guidelines of the infectious diseases working party (AGIHO) of the German society of hematology and oncology (DGHO). **Ann Hematol. 2003**; 82:S149-157.

6. Dugdale DC, Ramsey PG. *Staphylococcus aureus* bacteremia in patients with Hickman catheters. **Am J Med. 1990**; 89:137-141.

7. Bastani B, Minton J, Islam S. Insufficient penetration of systemic vancomycin into the PermCath lumen. **Nephrol Dial Transplant. 2000**; 15:1035-1037.

8. Maya ID, Carlton D, Estrada E, Allon M. Treatment of dialysis catheter-related *Staphylococcus aureus* bacteremia with an antibiotic lock: A quality improvement report. **Am J Kidney Dis. 2007**; 50:289-295.

9. Fernandez-Hidalgo N, Almirante B, Calleja R, Ruiz I, Planes AM, Rodriguez D, et al. Antibiotic-lock therapy for long-term intravascular catheter-related bacteraemia: Results of an open, non-comparative study. **J Antimicrob Chemother. 2006**; 57:1172-1180.

10. Poole CV, Carlton D, Bimbo L, Allon M. Treatment of catheter-related bacteraemia with an antibiotic lock protocol: Effect of bacterial pathogen. **Nephrol Dial Transplant. 2004**; 19:1237-1244.

11. Saxena AK, Panhotra BR, Naguib M, Sundaram DS, Venkateshhappa CK, Uzzaman W, et al. Vascular access related septicemia in hemodialysis: a focus on bacterial flora and antibiotic access salvage. **Saudi J Kidney Dis Transpl. 2002**; 13:29-34.

12. Mokrzycki MH, Zhang M, Cohen H, Golestaneh L, Laut JM, Rosenberg SO. Tunnelled haemodialysis catheter bacteraemia: risk factors for bacteraemia recurrence, infectious complications and mortality. **Nephrol Dial Transplant. 2006**; 21:1024-31.

13. Kim SH, Kang CI, Kim HB, Youn SS, Oh MD, Kim EC, et al. Outcomes of Hickman catheter salvage in febrile neutropenic cancer patients with *Staphylococcus aureus* bacteremia. **Infect Control Hosp Epidemiol. 2003**; 24:897-904.

14. Fowler VG Jr, Justice A, Moore C, Benjamin DK Jr, Woods CW, Campbell S, et al. Risk factors for hematogenous complications of intravascular catheter-associated *Staphylococcus aureus* bacteremia. **Clin Infect Dis. 2005**; 40:695-703.

15. Lesens O, Methlin C, Hansmann Y, Remy V, Martinot M, Bergin C, et al. Role of comorbidity in mortality related to *Staphylococcus aureus* bacteremia: a prospective study using the Charlson weighted index of comorbidity. **Infect Control Hosp Epidemiol. 2003**; 24:890-896.

16. Soriano A, Martinez JA, Mensa J, Marco F, Almela M, Moreno-Martinez A, et al. Pathogenic significance of methicillin resistance for patients with *Staphylococcus aureus* bacteremia. **Clin Infect Dis. 2000**; 30:368-373.

17. Jensen AG, Wachmann CH, Espersen F, Scheibel J, Skinhøj P, Frimodt-Møller N. Treatment and outcome of *Staphylococcus aureus* bacteremia: a prospective study of 278 cases. **Arch Intern Med. 2002;** 162:25-32.

18. Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, Cheng AC, Dudley T, Oddone EZ. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. **Arch Intern Med. 2003**; 163:2066-2072.

19. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. **J Clin Microbiol. 2003;** 41:3655-3660.

20. Melzer M, Eykyn SJ, Gransden WR, Chinn S. Is methicillin-resistant S*taphylococcus aureus* more virulent than methicillin-susceptible *S. aureus*? A comparative cohort study of British patients with nosocomial infection and bacteremia. **Clin Infect Dis. 2003**; 37:1453-1460.

21. Lodise TP, McKinnon PS, Swiderski L, Rybak MJ. Outcomes analysis of delayed antibiotic treatment for hospital-acquired *Staphylococcus aureus* bacteremia. **Clin Infect Dis. 2003**; 36:1418-1423.

22. Fätkenheuer G, Preuss M, Salzberger B, Schmeisser N, Cornely OA, Wisplinghoff H, et al. Long-term outcome and quality of care of patients with *Staphylococcus aureus* bacteremia. **Eur J Clin Microbiol Infect Dis. 2004**; 23:157-162.

23. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. **Lancet. 2004**; 364:703-705.

24. Kaech C, Elzi L, Sendi P, Frei R, Laifer G, Bassetti S, et al. Course and outcome of *Staphylococcus aureus* bacteraemia: A retrospective analysis of 308 episodes in a swiss tertiary-care centre. **Clin Microbiol Infect. 2006**; 12:345-352.

25. Kim SH, Park WB, Lee CS, Kang CI, Bang JW, Kim HB, et al. Outcome of inappropriate empirical antibiotic therapy in patients with *Staphylococcus aureus* bacteraemia: analytical strategy using propensity scores. **Clin Microbiol Infect. 2006**; 12:13-21.

26. Wang FD, Chen YY, Chen TL, Liu CY. Risk factors and mortality in patients with nosocomial *Staphylococcus aureus* bacteremia. **Am J Infect Control. 2008**; 36:118-122.

27. Rieg S, Peyerl-Hoffmann G, de With K, Theilacker C, Wagner D, Hübner J, et al. Mortality of *Staphylococcus aureus* bacteremia and infectious diseases specialist consultation – a study of 521 patients in Germany. **J Infect. 2009**; 59:232-239.

28. Ammerlaan H, Seifert H, Harbarth S, Brun-Buisson C, Torres A, Antonelli M, et al. The European Practices of Infections with *Staphylococcus aureus* (SEPIA) Study Group. Adequacy of antimicrobial treatment and outcome of *Staphylococcus aureus* bacteremia in 9 Western European countries. **Clin Infect Dis. 2009**; 49:997-1005.

29. Price J, Baker G, Heath I, Walker-Bone K, Cubbon M, Curtis S, et al. Clinical and Microbiological Determinants of Outcome in *Staphylococcus aureus* Bacteraemia. **Int J Microbiol. 2010**; 2010:654858.

30. Saad TF. Bacteremia associated with tunneled, cuffed hemodialysis catheters. **Am J Kidney Dis. 1999**; 34:1114-1124.

31. Rubin LG, Shih S, Shende A, Karayalcin G, Lanzkowsky P. Cure of implantable venous port-associated bloodstream infections in pediatric hematology-oncology patients without catheter removal. **Clin Infect Dis. 1999**; 29:102-105.

32. Kim SH, Kang CI, Kim HB, Youn SS, Oh MD, Kim EC, et al. Outcomes of Hickman catheter salvage in febrile neutropenic cancer patients with *Staphylococcus aureus* bacteremia. **Infect Control Hosp Epidemiol. 2003**; 24:897-904.

33. Pigrau C, Rodríguez D, Planes AM, Almirante B, Larrosa N, Ribera E, et al. Management of catheter-related *Staphylococcus aureus* bacteremia: when may sonographic study be unnecessary? **Eur J Clin Microbiol Infect Dis. 2003**; 22:713-719.

34. Maya ID, Carlton D, Estrada E, Allon M. Treatment of dialysis catheter-related *Staphylococcus aureus* bacteremia with an antibiotic lock: a quality improvement report. **Am J Kidney Dis. 2007**; 50:289-295.

35. Park KH, Cho OH, Lee SO, Choi SH, Kim YS, Woo JH, et al. Outcome of attempted Hickman catheter salvage in febrile neutropenic cancer patients with *Staphylococcus aureus* bacteremia. **Ann Hematol. 2010**; 89:1163-1169.

IX

Isolation, structural characterization and activity of epilancin 15X, a novel lantibiotic from a clinical strain of *Staphylococcus epidermidis*

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Abstract

The potential application of lantibiotics as food-preserving agents and more recently as antibiotics has strongly increased the interest in these antibacterial peptides. Here, we report the elucidation of the primary and three-dimensional structures of the novel lantibiotic epilancin 15X from *Staphylococcus epidermidis* using high-resolution nuclear magnetic resonance spectroscopy and tandem mass spectrometry. The molecule contains ten post-translationally modified amino acids, three lanthionine ring structures and a hydroxy-propionyl N-terminal moiety. The primary and tertiary structure and the distribution of positive charges are closely similar to the previously identified lantibiotic epilancin K7, most likely indicative of a common mode of action. However, epilancin 15X is active against enterococci, whilst epilancin K7 is not.

Introduction

Lantibiotics is the name for a group of bacterial peptides with antibacterial action, which are ribosomally synthesized as prepeptides and post-translationally modified to contain the dehydrated amino acids a,b-didehydroalanine (Dha) and a,b-didehydrobutyric acid (Dhb) as well as the thioether bridges formed by lanthionine (Lan) and b-methyllanthionine (MeLan)[1-3] (see also Figure 1). Nisin, a member of the lantibiotic family, has been commonly used as a food preservative (known as E234) for its strong activity against food pathogens.[4] Recently, the application of lantibiotics, as antibiotic reagents, has moved a big step forwards as the structure of nisin in complex with its natural target lipid II revealed a possible common motif that may pave the way to the design of novel antibiotics. The potential application of other lantibiotics as food preservatives or as antibiotics has led so far to the discovery of over 40 different lantibiotics, produced by bacteria isolated from different sources.

Lantibiotics are sub-divided into type A and type B peptides. Type A lantibiotics are flexible, cationic and elongated peptides which exert their function by pore formation in the bacterial membrane, whereas type B are generally more rigid globular molecules that act by disruption of enzyme functions such as peptidoglycan biosynthesis and phospholipase activity.[2]

The lantibiotic described in this paper is produced by *Staphylococcus epidermidis* 15X154, a clinical strain isolated from a wound. *S. epidermidis* is a Gram-positive, katalase positive and coagulase negative coccus and is a colonizer of the human skin. However, it may cause serious infections in immunocompromised patients and in patients with indwelling devices.[6] Thus far, four lantibiotic peptides produced by *S.epidermidis* strains have been identified, namely pep5,[7] epidermin,[8] epicidin 280,[9] and epilancin K7,[10,11] all of which are type A lantibiotics.

Structural characterization of novel lantibiotics remains a challenge, primarily due to the problematic designation of the thioether bridges.[3] Nuclear magnetic resonance (NMR) spectroscopy is often employed to complement DNA sequencing in order to obtain a definitive primary sequence. In this paper, we combined NMR spectroscopy and nano-scale

liquid chromatography/tandem mass spectrometry (MS) to determine the primary sequence and molecular structure of the newly isolated active peptide. The peptide was found to highly resemble the previously identified epilancin K7[10,11] with 68% sequence identity, three nearly identical lanthionine rings and a modified amino acid at the N-terminus. This prompted us to name the new lantibiotic epilancin 15X.

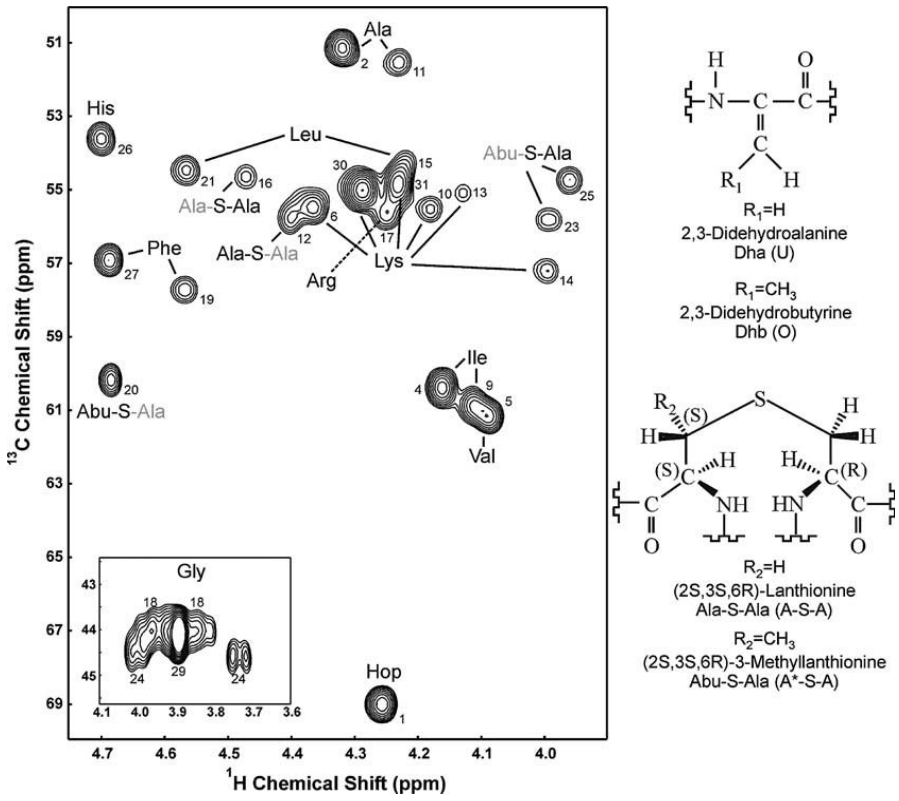

Figure 1: The 1Ha–13Ca region of a natural abundance 1H–13C-HSQC spectrum of epilancin 15X. Peaks are labeled with the corresponding residue number and identity. The inset shows the glycine region. The structure of the post-translational modified residues is shown in the right. The N-terminus is a Hop group indicated as Hop.

Materials and methods

2.1. Discovery, isolation and purification of the active peptide.

Over 1000 clinical bacterial strains from the strain bank of the European Network of Antimicrobial Resistance and Epidemiology (ENARE) were tested on Mueller Hinton agar for direct inhibitory activity against a range of multi-resistant pathogens. From strains which exhibited direct activity supernatants were tested against the inhibited pathogens. Finally, from all tested strains, *S. epidermidis* strain 15X154 was selected because of the reproducible and stable effect of its supernatant against meticillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Strain 15X154 was probably a contaminant in the strain bank, as the strain bank description only mentioned an *Enterococcus faecalis* isolate (15D154) from the wound.

For isolation, 1.5 liters of Mueller Hinton broth were inoculated with 100 µl of overnight *S. epidermidis* 15X154 culture and incubated for 16 h at 37º C on a shaker. After centrifugation, the supernatant was filter sterilized and purified on an Äkta FPLC system (Amersham, Uppsala, Sweden) by cation exchange chromatography (HiTrap SP XL), hydrophobic interaction chromatography (Source 15 PHE) and reverse phase chromatography (Source 15 RPC, Amersham), essentially as described previously.[12] Activity in the FPLC fractions was determined as described previously,[13] using *Staphylococcus aureus* strain 29213 as the indicator strain. The purified compound was stored at 4º C.

2.2. NMR spectroscopy

Two-dimensional homonuclear 1H total correlation spectroscopy (TOCSY) and nuclear Overhauser enhancement spectroscopy (NOESY) spectra were recorded on a Bruker DRX 750 MHz spectrometer at 305 and 283 K with 1.25 mg of epilancin 15X dissolved in 500 µl of 90% H_2O/10% D_2O, 10 mM d3-sodium acetate at pH 4. Mixing times were 100, 200 and 300 ms in the NOESY experiments and 80 ms in the TOCSY experiments. Natural abundance 1H–13C heteronuclear single-quantum correlation (HSQC) and 1H–13C heteronuclear multiple bond correlation (HMBC) NMR spectra were acquired on a Bruker

Avance 600 MHz spectrometer equipped with a cryoprobe system at 283 K with 3 mg epilancin 15X in 300 µl D_2O using a Shigemi tube. The 1H and 13C spectral widths were 10 ppm and 150 or 200 ppm, respectively. All spectra were processed using NMRPipe[14] and analyzed using NMRView.[15] The assigned chemical shifts were deposited in BioMagResonBank under Accession No. 6352.

2.3. Structure calculation and analysis

Structure calculations were performed with CNS[16] using the ARIA setup and protocols.[17] Most peaks in the NOESY spectra were unambiguously assigned, except for those that show spectral overlap, which were assigned as ambiguous with a lower weighing factor. The semi-automated NOE assignment with ARIA and additional parameters and topologies introduced to define Dha, Dhb, MeLan and Lan were done as described previously.[18] The topology for hydroxy-propionyl (Hop) was constructed based on alanine with comparison of available databases. Three thioether bridges between residues 12 and 16 (ring A), 20 and 23 (ring B) and 22 and 25 (ring C) were introduced. A simulated annealing protocol was performed using torsion angle dynamics as described before.[18] All 100 structures were subjected to explicit solvent refinement[19] and the 20 lowest-energy structures were kept for structural analysis. The structural coordinates were deposited in the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) under Accession Code 1W9N.

2.4. Mass spectrometry

Nano-scale liquid chromatography MS and tandem MS (MS/MS) were performed on a 1100 series liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA) and a Q-Tof Ultima API hybrid quadrupole/time of flight mass spectrometer (Waters corporation, Milford, MA, USA) as described previously.[20] Samples were diluted to 1 fmol/µl in 5% DMSO/5% formic acid and 10 µl were injected onto a C18 trapping column. Analyte separation was performed on a C18 column using a linear gradient from 0% to 60% acetonitril + 0.1M acetic acid (both columns manufactured by Nano-separations, Nieuwkoop, The Netherlands). Data were analyzed using the MassLynx 3.4 140 software.

2.5. Peptide digestion

The peptide was hydrolyzed with modified trypsin according to the procedure provided by the supplier (Boehringer Mannheim, Germany). Approximately 100 pmol of peptide was incubated with 20 pmol enzyme in a buffer solution (100 µl) for 4 h at 37º C. Digestion was terminated by acidification with 1 µl of acetic acid.

2.6. Antibiotic peptides and vancomycin

Epilancin K7 and pep5 were kindly provided by Dr. Bierbaum (Institute of Medical Microbiology and Immunology, University of Bonn, Bonn, Germany). Vancomycin was purchased from Eli Lilly and co. (Indianapolis, IN).

2.7. Susceptibility testing

Minimal inhibitory concentrations (MIC's) were determined for epilancin 15X, epilancin K9, pep5 and vancomycin. Susceptibility testing was performed by microdilution, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) document M7-A7. Three to five colonies of the tested micro-organisms were selected from an overnight culture on TSA agar w/ sheep blood and suspended with a sterile swab in 0.9% NaCl to a density of 0.5 McFarland, and this suspension was diluted 1:500 in cation-adjusted Mueller Hinton (MH) broth. 50 µl of the bacterial suspension in MH broth was added to 50 µl of serial dilutions of the tested antimicrobial substances in 96-well round bottom plates, and incubated overnight at 35˚ C. After a minimum of 18 hours incubation, the plates were read visually and the MIC was determined as the lowest concentration at which no visible growth of the bacteria occurred.

For the assays 58 strains were selected from strain bank of ENARE: 14 MRSA, 15 methicillin-susceptible *Staphylococcus aureus* (MSSA), 16 vancomycin-susceptible enterococci (VSE), and 13 VRE.

Results

3.1. Isolation and purification

Pure epilancin 15X was obtained in a three-step liquid chromatography setup as often described for lantibiotics.[9,21] The yield was typically 0.5 mg peptide/l culture. Antibiotic activity remained stable during the purification process and partly purified peptide solutions could be stored for several weeks at 4º C without significant loss of activity.

3.2. Spin system analysis & sequence determination by NMR

The number and nature of residues of the peptide was determined using the 2D TOCSY and NOESY spectra recorded at 283 K. No signs of spin diffusion were observed in the 300 ms NOESY spectrum so it was used for the structure calculation. For initial identification of each spin system, chemical shifts were compared with random coil values.[22] A similar approach was applied for the modified amino acids by comparison with other lantibiotics.[5,10,23,24] Sequential assignment was achieved using standard NOE connectivity-based protocol.[25] Spectral overlaps were resolved by inspection of either homonuclear NMR spectra at 305 K or by the 13C heteronuclear spectra. All residues could be unambiguously identified and sequentially assigned in this way. The results were then confirmed by the 1H–13C HMBC experiment. In addition, the combined use of the 1H–13C HSQC and 1H–13C HMBC spectra enabled complete 13C chemical shift assignment of the backbone Ca and side chain carbon atoms, and 87% of the carbonyl atoms.

Figure 1 shows the assigned 1Ha–13Ca region of the 1H–13C HSQC. The observation of the Ala2 amide proton (which was first believed to be the N-terminal residue) led to the idea of an additional residue located prior to it. Comparison with Pep5, epicidin 280 and epilancin K7[26] and close inspection of all NMR spectra in conjunction with the molecular weight obtained from MS (see the following section) allowed us to unambiguously assign the N-terminus to Hop. Analysis of the NOE connectivities between the residue pairs of the lanthionine rings allowed for the localization of the thioether bridges. The combined analysis of all NMR data established the primary structure of the active

peptide with a calculated mass of 3173 Da: a total number of 31 residues, including 10 modified amino acids, three lanthionine rings (denoted as A, B and C in Figure 2) and Hop at the N-terminus.

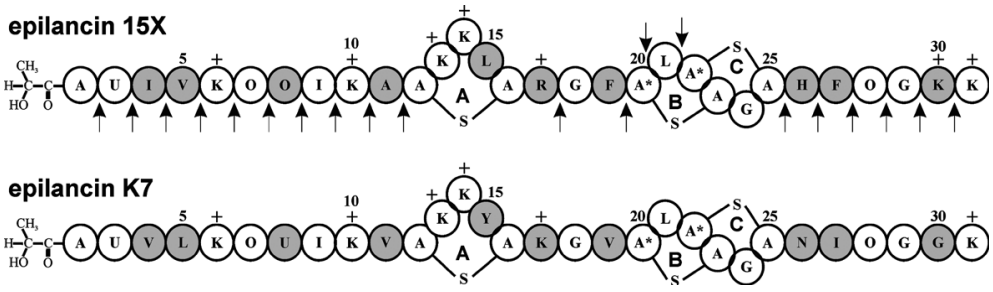

Figure 2: Primary structure of epilancin 15X compared to that of epilancin K7. Lanthionine rings A, B and C are indicated. The residues that are different between the two lantibiotics are shaded grey. The positively charged residues are indicated by a plus sign. The cleavage sites that were detected by MS are indicated by arrows.

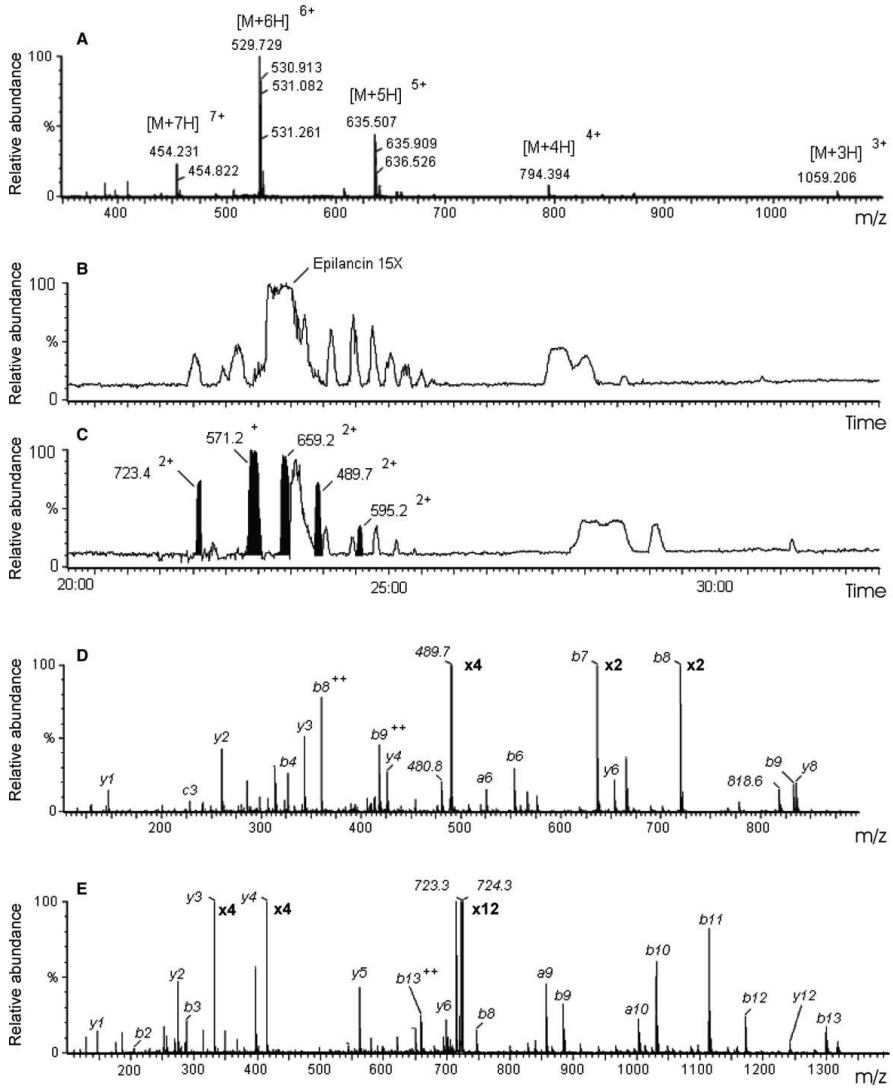

Figure 3: Analysis of epilancin 15X by MS. (A) Mass spectrum of epilancin 15X. The $[M + 6H]^{6+}$ ion (m/z ratio 529.729) is most abundant. (B) Chromatogram of epilancin 15X and (C) of the peptide digest fragments. After digestion, five new peaks can be retrieved. (D) MS/MS spectra of fragments 1–10 (489.72+) and (E) of 18–31 (723.42+). Fragment ions are marked.

3.3. Analysis by MS

In order to verify the sequence obtained by NMR, mass spectra of digested and undigested peptide samples were analyzed by MS. The mass spectrum of the undigested peptide yielded an envelope of multiple charges ranging from 3 to 7 charges, the $[M + 6H]^{6+}$ ions with a m/z of 529.7 being the most abundant (Figure 3A). A molecular weight of 3172.9 Da was calculated, which is in agreement with the mass determined from the NMR-derived sequence. In the analysis of the tryptic digest, five major fragments were detected (Figures 3B and C). These ions were selected for MS/MS analysis (Figures 3D and E) and were found to correspond to residues 18–31, 1–5, 18–30, 1–10 and 18–29, respectively. No digestion fragment corresponding with residues 11–17 could be identified. In the MS/MS spectrum of the 1–5 digest fragment, a- and b-ions were accompanied by ions at 14 Da lower m/z value, which can be explained by the loss of a CH2 group by the Hop moiety. As observed for other lantibiotics,[27,28] little cleavage occurred in the part of the molecule containing two MeLan rings (between Abu20 and Ala25). Ion masses of 288.1 and 401.2 were found in the spectra of all three digest fragments 18–29, 18–30 and 18–31 corresponding to the b3 and b4-ions formed by cleavage between Abu20 and Leu21, and between Leu21 and Abu22, respectively. The mass difference of 83 Da between the b2 (205.1) and b3-ion suggests loss of a hydrogen atom and formation of Dhb from Abu after cleavage of the thioether bridge. The cleavage sites retrieved by MS/MS are indicated in Figure 3. A total of 21 out of 30 expected cleavage sites (70%) could be confirmed for epilancin 15X.

3.4. The 3D solution structure of epilancin 15X and comparison to epilancin K7

The solution structure of epilancin 15X was determined based on the NOE-derived distance restraints (Figure 4). Like many type A lantibiotics, it exhibits no conventional secondary structure but well-defined local ring structures. The temperature dependency of amide proton chemical shifts (temperature coefficient) provides information on the 233
involvement in hydrogen bond formation or sequestering from the solvent and has been used in studies of other lantibiotics.[11,29] The temperature coefficients of individual amide protons were measured (see Figure 5) and compared with those measured for epilancin K7.[11]

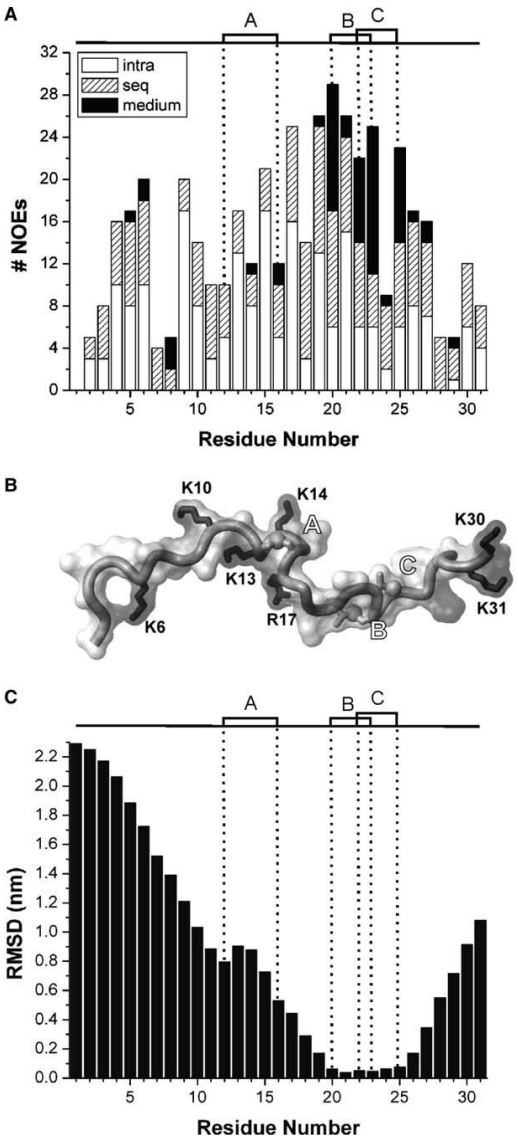

Figure 4: The three-dimensional solution structure of epilancin 15X. (A) Distribution of NOEs along the sequence divided into intra-residual (white bars), sequential (hashed bars) and medium range NOEs (black bars). Note that the most medium-range NOEs were identified for residues in rings B and C. (B) A combined ribbon/surface presentation of the lowest-energy structure. The side chains of the positively charged residues are shown in stick representation with the corresponding residue numbers. The sulfur atoms in the three rings A, B and C are shown in spheres. The figure was generated using MolMol .[33] (C) The main-chain RMS displacements from the mean structure of the ensemble of 20 lowest-energy structures showing that the structure is mostly well-defined for rings B and C, in agreement with the larger number of medium-range NOEs observed in this region (panel A). The three ring-systems A, B and C are indicated at the top of panels (A) and (C).

Generally, in the regions of the ring structures, the lower temperature coefficients correlate with the lower surface accessibility of the amide protons that was calculated from the ensemble of 20 structures (data not shown). The sequential profiles of the temperature coefficients of the two homologous lantibiotics are very similar, indicating that epilancin 15X resembles epilancin K7 also in 3D structure. Both molecules are also highly positively charged (about 20% of the total number of amino acids) and the distribution of these charges are almost identical except for the missing charge at the C-terminus in epilancin K7 (see Figures 2 and 4B). The homology between epilancin K7 and epilancin 15X suggests that they may be natural variants, as is the case for nisin A and nisin Z.[30,31]

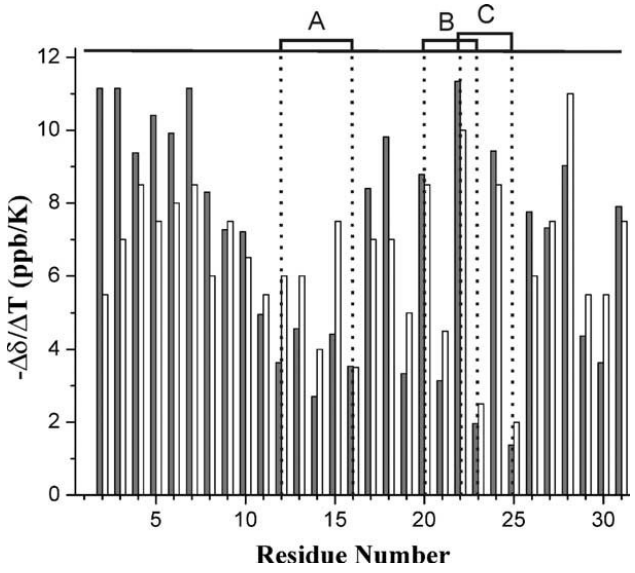

Figure 5: NMR-derived structural data for epilancin 15X and epilancin K7. The temperature-coefficients (Dd/DT) of epilancin 15X were derived from TOCSY experiments at 283 and 305 K, and are shown by dark-grey bars. The temperature-coefficients of epilancin K7[11] are shown by white bars. Note the typical up-down pattern for the residues in rings B and C in both peptides. The three ring-systems A, B and C are indicated at the top of the figure.

3.5. Antibiotic activity

Preliminary testing found epilancin 15X active against Gram-positive micro-organisms but not Gram-negative micro-organisms. Epilancin 15X was therefore compared with two other lantibiotics – epilancin K7 and pep5 – and with the glycopeptide vancomycin for activity against the two most relevant multi-resistant Gram-positive pathogens: MRSA and VRE, as well as against meticillin-susceptible *S. aureus* (MSSA) and vancomycin-susceptible enterococci (VSE) (Table 1). Interestingly, although the activity of the three lantibiotics against *S. aureus* was comparable, epilancin 15X was the only lantibiotic active against enterococci, with MIC_{50} and MIC_{90} of 4 mg/l and 8 mg/l respectively. Epilancin K7 and pep5 had MICs over 32 mg/l for all enterococci. MIC_{50} and MIC_{90} of epilancin 15X against *S. aureus* were 1 µg/ml and 2 µg/ml respectively. MICs of coagulase-negative staphylococci are in general tenfold lower than those of *S. aureus* (data not shown).

1_A: Meticillin-susceptible *Staphylococcus aureus*

MIC (mg/l):	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>32**
Epilancin 15X		1	7	7					
Epilancin K7			3	5	3	2	1	1	
Pep5					1	3	9		2
Vancomycine	2	10	3						

1_B: Meticillin-resistant *Staphylococcus aureus*

MIC (mg/l):	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>32**
Epilancin 15X		1	1	2	8	1	1			
Epilancin K7	1			2	4	5				1
Pep5				1		2	2	5	2	2
Vancomycine			5	8	1					

1_C: Vancomycin-susceptible enterococci

MIC (mg/l):	0.25	0.5	1	2	4	8	16	32	>32
Epilancin 15X			1	2	6	6		1	
Epilancin K7									16
pep5									16
Vancomycine	4	5	1	3	1	2			

1_D: Vancomycin-resistant enterococci

MIC (mg/l):	1	2	4	8	16	32	>32
Epilancin 15 X	1	2	5	5			
Epilancin K7							13
Pep5							13
Vancomycin							13

Table 1: Minimal inhibitory concentrations (MICs) of epilancin 15X, epilancin K7, pep5 and vancomycin for (A) meticillin-susceptible *Staphylococcus aureus*, (B) meticillin-resistant *S. aureus*, (C) vancomycin-susceptible enterococci, and (D) vancomycin-resistant enterococci.

Discussion

Due to the presence of post-translationally modified amino acids, a reliable determination of lantibiotic amino acid sequence and secondary structure depends on a combination of techniques, usually DNA sequencing combined with NMR spectroscopy. Initial difficulties with DNA sequencing, which are not uncommon with lantibiotics, prompted us to use MS, which was especially helpful in the identification of the N-terminal Hop. The molecular mass as measured by MS matched the mass derived by NMR. In addition, MS/MS was capable of

confirming several (stretches of) amino acid residues. NMR and MS were also successfully applied previously to determine the primary and tertiary structure of the nonadecapeptide cinnamycin, a type B lantibiotic.[32] The high similarity in primary and tertiary structure as well as in the distribution of positive charges, most likely reflects a similar way in which epilancin K7 and the novel lantibiotic epilancin 15X recognize their targets.

However, differences in spectrum of activity between the different lantibiotics exist: only epilancin 15X is active against enterococci. This activity may explain why we were able to recover *S. epidermidis* strain 15X154 from the vial in which *Enterococcus faecalis* strain 15D154 was stored.

In attempts to develop novel antibiotics against multi-drugresistant micro-organisms the lantibiotics have gained interest, due to their specific mode of action which is different from all currently employed antibiotics in human medicine. Although the activity of epilancin 15X interesting application of the substance as an antibiotic is hampered by many obstacles. At the moment, synthetic production of lantibiotics is not feasible and isolation from bacterial supernatant is limited by the low concentrations produced. (For instance, *S. epidermidis* 15X154 is actually itself inhibited by epilancin 15X concentrations of 1 mg/l or higher, and does therefore never produce more than 0,5 mg per liter.) Furthermore, the molecular size of lantibiotics, often over 3 kDa, indicates that systemic therapy will only be possible by intravenous infusion and makes them prone for eliciting immunological responses.

However, as mentioned above, epilancin 15X apparently has specific structural properties that render it active against enterococci. Therefore, even if the substance itself will never be used as as antibiotic, analysis of its structure may yield leads for the development of more suitable drugs.

References

1. Schnell N, Entian KD, Schneider U, Gotz F, Zahner H, Kellner R, et al. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. **Nature 1988**; 333:276–278.

2. McAuliffe O, Ross RP, Hill C. Lantibiotics: Structure, biosynthesis and mode of action. **FEMS Microbiol Rev 2001**; 25:285–308.

3. Xie L, van der Donk WA. Post-translational modifications during lantibiotic biosynthesis. **Curr Opin Chem Biol 2004**; 8:498–507.

4. Delves-Broughton J, Blackburn P, Evans RJ, Hugenholtz J. Applications of the bacteriocin, nisin. **Antonie Van Leeuwenhoek 1996**; 69:193–202.

5. Hsu STD, Breukink E, Tischenko E, Lutters MA, de Kruijff B, Kaptein R, et al. The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. **Nature Struct Mol Biol 2004**; 11:963–967.

6. Archer GL. *Staphylococcus epidermidis* and other coagulase-negative staphylococci in: **Principles and Practice of Infectious Diseases 2000**; pp. 2092–2100

7. Sahl HG, Grossgarten M, Widger WR, Cramer WA, Brandis H. Structural similarities of the staphylococcinlike peptide Pep-5 to the peptide antibiotic nisin. **Antimicrob Agents Chemother 1985**; 27:836–840.

8. Allgaier H, Jung G, Werner RG, Schneider U, Zahner H. Epidermin: sequencing of a heterodetic tetracyclic 21-peptide amide antibiotic. **Eur J Biochem 1986**; 160:9–22.

9. Heidrich C, Pag U, Josten M, Metzger J, Jack RW, Bierbaum G, et al. Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster. **Appl Environ Microbiol 1998**; 64:3140–3146.

10. van de Kamp M, Horstink LM, van den Hooven HW, Konings RN, Hilbers CW, Frey A, et al. (1995) Sequence analysis by NMR spectroscopy of the peptide lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7. **Eur J Biochem 1995**; 227:757–771.

11. van de Kamp M, van den Hooven HW, Konings RN, Bierbaum G, Sahl HG, Kuipers OP, et al. Elucidation of the primary structure of the lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7. Cloning and

characterisation of the epilancin-K7-encoding gene and NMR analysis of mature epilancin K7. **Eur J Biochem 1995**; 230:587–600.

12. Nissen-Meyer J, Holo H, Håvarstein LS, Sletten K, Nes IF. A novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides. **J Bacteriol 1992**; 174:5686–5692.

13. Cintas LM, Rodriguez JM, Fernandez MF, Sletten K, Nes IF, Hernandez PE, et al. Isolation and characterization of pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. **Appl Environ Microbiol 1995**; 61:2643–2648.

14. Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. NMRPipe: A multidimensional spectral processing system based on UNIX pipes. **J Biomol NMR 1995**; 6:277–293.

15. Johnson BA, Blevins RA. NMRview: A computer program for the visualization and analysis of NMR data. **J Biomol NMR 1994**; 4:603–614.

16. Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, et al. Crystallography & NMR system: A new software suite for macromolecular structure determination. **Acta Crystallogr D Biol Crystallogr 1998**; 54:905–921.

17. Nilges M, Donoghue SI. Ambiguous NOEs and automated NOE assignment. **Prog Nucl Magn Reson Spectrosc 1998**; 32:107–139.

18. Hsu ST, Breukink E, Bierbaum G, Sahl HG, de Kruijff B, Kaptein R, et al. NMR study of mersacidin and lipid II interaction in dodecylphosphocholine micelles. Conformational changes are a key to antimicrobial activity. **J Biol Chem 2003**; 278:13110–13117.

19. Linge JP, Williams MA, Spronk CA, Bonvin AM, Nilges M. Refinement of protein structures in explicit solvent. **Proteins 2003**; 50:496–506.

20. Meiring HD, van der Heeft E, ten Hove GJ, de Jong AP. Nanoscale LC–MS(n): Technical design and applications to peptide and protein analysis. **J Sep Sci 2002**; 25:557–568.

21. Mørtvedt CI, Nissen-Meyer J, Sletten K, Nes IF. Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45. **Appl Environ Microbiol 1991**; 57:1829–1834.

22. Wishart DS, Nip AM. Protein chemical shift analysis: A practical guide. **Biochem Cell Biol 1998**; 76:153–163.

23. Chan WC, Bycroft BW, Leyland ML, Lian LY, Yang JC, Roberts GC. Sequence-specific resonance assignment and conformational analysis of subtilin by 2D NMR. **FEBS Lett 1992**; 300:56–62.

24. Kuipers OP, Rollema HS, Yap WM, Boot HJ, Siezen RJ, de Vos WM. Engineering dehydrated amino acid residues in the antimicrobial peptide nisin. **J Biol Chem 1992**; 267:24340–24346.

25. Wüthrich K. **NMR of Proteins and Nucleic Acids, 1986**.

26. Sahl HG and Bierbaum G. Lantibiotics: Biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. **Annu Rev Microbiol 1998**; 52:41–79.

27. Lavanant H, Derrick PJ, Heck AJ, Mellon FA. Analysis of nisin A and some of its variants using Fourier transform ion cyclotron resonance mass spectrometry. **Anal Biochem 1998**; 255:74–89.

28. Kleinnijenhuis AJ, Duursma MC, Breukink E, Heeren RM, Heck AJ. Localization of intramolecular monosulfide bridges in lantibiotics determined with electron capture induced dissociation. **Anal Chem 2003**; 75:3219–3225.

29. Hsu ST, Breukink E, de Kruijff B, Kaptein R, Bonvin AM, van Nuland NA.Mapping the targeted membrane pore formation mechanism by solution NMR: The nisin Z and lipid II interaction in SDS micelles. **Biochemistry 2002**; 41:7670–7676.

30. de Vos WM, Mulders JW, Siezen RJ, Hugenholtz J, Kuipers OP. Properties of nisin Z and distribution of its gene, nisZ, in Lactococcus lactis. **Appl Environ Microbiol 1993**; 59:213–218.

31. Sahl HG, Jack RW, Bierbaum G. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. **Eur J Biochem 1995**; 230: 827–853.

32. Jung G, Sahl HG. **Nisin and Novel Lantibiotics 1991**; pp. 76–90.

33. Koradi R, Billeter M, Wüthrich K. MOLMOL: A program for display and analysis of macromolecular structures. **J Mol Graph 1996**; 14:29–32.

X

General discussion and future directions

In this thesis risk factors and management options for healthcare-associated infections with *Staphylococcus aureus* were explored.

The impact of healthcare-associated (HA-)infections – in the past usually referred to as nosocomial or hospital-acquired infections[1] – is dramatic, both on the individual patient and on healthcare statistics. In the United States, yearly an estimated 1.7 million patients acquire an HA-infection, which lead to 100.000 attributable deaths (data 2002).[2] A Belgian report calculated a yearly number of 125.000 HA-infections and 2600 deaths for that country.[3] Numbers and figures for the Netherlands are not available, but a large national study published in 2007 with hospital data from 2004 estimated that in 21% of 1700 yearly preventable deaths in hospitalized patients (i.e. 357 patients) a healthcare-associated infection was at least partially the cause.[4] In this report, however, most HA-infections were deemed "non-preventable complications", hereby suggesting that the total mortality is several times higher.

Furthermore, as a result of medical progress, HA-infections are on the rise. Novel therapies – such as monoclonal antibodies, cardiac assist devices, stem cell transplantations and extracorporeal membrane oxygenation, to name but a few – create new groups of patients at an increased risk of HA-infections. Prevention, early detection and treatment of HA-infections have become cornerstones in "best medical practice".

Finally, antibiotic resistance has the highest impact on those infections which are healthcare-associated: the high antibiotic use in hospital environments exerts an evolutionary pressure towards higher resistance. Pathogens are increasingly resistant and, therefore, more difficult to treat; omniresistant pathogenic bacteria no longer constitute science fiction. Their emergence also has an impact on the empiric antibiotic therapies, often forcing towards the use of agents which are less effective, less safe, and more expensive.

In classic microbiology and infectious diseases, a distinction was made between "obligate pathogens" i.e. micro-organisms that cannot colonize without causing an infection, "facultative pathogens" which may or may not cause infection, and "opportunistic

pathogens" which only infect immunocompromized hosts. This distinction, if at all valid, does not apply to HA-infections. Here, whether or not a micro-organism is a "pathogen" depends primarily on host factors, and not on the micro-organism. This is exemplified by the case report in **chapter III**, which describes an endocarditis caused by a strain of MRSA which is normally only found as a colonizer of farm animals. In this specific case, however, due to the underlying disease of the patient a severe infection developed, probably through hematogenic seeding of the bacterium from an intravascular catheter.

Colonization always precedes infection in healthcare-associated infections. Whether or not colonization progresses into infection is dependent on the bacterium, the host and the circumstances, but if one thing has become apparent in this thesis, it is that care should be taken before regarding *S. aureus* as a mere colonizator. *S. aureus* belongs in the nares (and even there it may not be completely harmless), not in wounds or attached to indwelling devices. Anywhere the integrity of our body is breached, an entry site is created, and once the bacterium has settled it is just a question of time before an infection develops. This holds true for urinary tract colonization (**chapter IV**), for colonization of neurosurgical drains (**chapter V**), and for colonization of intravascular catheters (**chapters VII and VIII**). Furthermore, an infection may even develop after removal of a colonized drain or catheter (**chapters V, VII and VIII**).

Many risk factors for HA-infections have been elucidated, and quite some preventive interventions have been described, targeted at different phases in the development of infection. Some of which are of general relevance, and some of which specifically target *S. aureus*. The foremost are described below:

1. Outbreak management

The most secure way of preventing infection with a micro-organism is preventing every contact with this micro-organism. Although it is sometimes suggested that infections with hospital flora simply supplant those with the patients' original flora, they probably are for the

larger part additive. This is most obvious for infections by faecal pathogens such as *Clostridium difficile* and norovirus – infections which normal patient flora would never cause – but has recently also been demonstrated for MRSA. In hospitals where MRSA over the years has become endemic, the number of MSSA-bacteremias remained at a constant level: hospitals with endemic MRSA simply experienced an increase in the number of bacteremia's.[5] Future studies will need to evaluate whether infections with micro-organisms such as amoxycillin-resistant *Enterococcus faecium* clonal complex 17,[6] ESBL *E. coli*,[7] carbapenemase-positive Klebsiella pneumoniaea,[8] multi-drug resistant enterobacter,[9] multi-drug resistant *Acinetobacter baumannii* (like the "talibacter")[10] are replacements of ordinary infections or additional infections.

Outbreak management requires interest, effort, and instruments, and it is most effective in an early stage, when awareness may still be low or non-existent. Once problems become obvious, the situation may have become irreversible.

2. Treatment of carriers and bacterial load reduction / de-colonization

If acquisition of *S. aureus* has already occurred, eradication of the bacterium or significant reduction of the bacterial load is the next logical step in the prevention of HA-infections. Nasal carriage of *S. aureus* has been identified as a risk factor for post-operative infections, bowel colonization as even more so, and in **chapter IV** we found that urinary colonization with *S. aureus* is often followed by bacteremia. The "weight of the *S. aureus* load" probably predicts the chances of trouble.

In *S. aureus* carriers, eradication of the bacterium with mupirocin applied to the nares has been demonstrated to reduce the post-operative infectious complications of certain procedures.[11] The high contribution of *S. aureus* to the total number of HA-infections is thus high, that screening for the bacterium prior to treatment is not cost-effective and even counter-productive. Economic principles favor treatment of all patients with mupirocin.[12]

Another example of bacterial load reduction is the application of mupirocin around the peritoneal catheter insertion sites in dialysis patients. This has been shown to be an effective method to prevent of exit site infections.[13] Also the application of selective decontamination of the digestive tract (SDD) – which is associated with approximately a 3% reduction in ICU-mortality – is based on this principle.[14]

3. Hygiene measures and prophylaxis during procedures

Hospitalized patients colonized with *S. aureus* are especially at risk of infection after any invasive procedure. It is broadly recognized that such complications may be prevented by adherence to protocols and guidelines, although evidence on the matter is often quite "soft". Evaluations of implementation/intervention-studies in infection prevention suffer heavily from publication-bias and from "regression to the mean". Numbers of HA-infections fluctuate over time, and measures are generally taken when the incidence is at a high point—the moment at which the incidence is expected to drop simply by the natural course of events. In order to truly establish a significant effect, multiple measurements need to be taken before and after the intervention, but very few studies comply with this prerequisite.

The fact that certain preventive measures may decrease development of CRBSI is especially apparent when comparing the high rate of CRBSI caused by intravascular lines placed urgently when compared with those placed electively.[15] Guidelines for prevention of CRBSI have been developed, for instance by the CDC and the IDSA,[16] with evidence-based recommendations, but to a larger degree with common sense-based recommendations.

It has been demonstrated that adhering to protocols during operative procedures significantly (and relevantly) decreases infectious complications. Particularly in orthopedic implant surgery, methods that minimize bacterial contamination, and in particular adequate antibiotic prophylaxis, have led to important reductions in infectious complications.[17]

Unfortunately, it is common for adherence to guidelines to decrease steadily after policy-interventions. Therefore, constant attention, reminders, and action are warranted to procure their effectivity, also at times when problems appear marginal and irrelevant.

4. Antibiotic- and antiseptic-coated catheters and implants

Absolute sterility is not feasible, neither in surgical procedures nor in the placement of indwelling catheters. The next chronological step in prevention, after maximum effort to procure asepsis, would be the use of devices which themselves impede colonization.

Antibiotic-coated catheters (either with rifampicin + minocycline or with silver sulfadiazine + chlorhexidine) have been evaluated in many randomized, open trials. However, whether or not these offer an advantage has not been definitely answered, mainly because all studies thus far have been severely underpowered. Assuming an average time in situ of 10 days for an IV-catheter, an expected number of 4 CR-BSIs / 1000 catheter days (reports vary between 1.2 and 5.7),[18] and an expected reduction in episodes of 50%: a total of 1173 patients would be needed in both the intervention and control group to achieve 80% power; for a reduction of 75% still a total of 870 patients should be included. The largest published study to date, however, included only 738 patients.[19] Considering the expenses involved in the use of coated catheters, routine implementation cannot possibly be considered before a true effect is established,[20] and before publication bias has been ruled out as the sole responsible factor for the effects reported thus far. Finally, it is needed to point out that most trials on the subject use surrogate end-points, such as catheter colonization instead of CRBSI, or do not have clear-cut definitions for this infection (most even accept a single positive blood culture with CoNS as proof of CRBSI),[21] and that most studies on the subject have been performed by the inventors themselves.[22] A large independent multicenter trial already proposed and funded in 2004[22] has somehow disappeared with no traces on www.clinicaltrials.gov, but two other trials *are* registered and expected to provide results in

the near future. The estimated enrolments are 1200 and 580 patients (the latter, thus, still underpowered).

In 2009 a rifampicin + minocycline-coated penile implant was FDA-approved; in a retrospective study of over 6000 implants infection rates were significantly lower with antibiotic coating (0.3% vs. 1.6%).[23] Other applications – such as orthopedic implants[24] and implantable cardiac devices[25] – are still in early experimental phases[26] and their routine use is far away.

5.1 Management of colonized intravascular catheters and early treatment of CA-SAB

Prevention does not stop once an infection is established. Secondary prevention – to detect and treat a disease in an early stage – is equally important. **Chapters VIIa and VIIb** focused on secondary prevention of intravascular infections: preventing that an infected / colonized catheter entry site develops into a CA-SAB.

CVC-colonization with *S. aureus* occurs mainly cutaneously, with the bacterium colonizing from the catheter exit site inward. Once the catheter has been colonized up to the tip, it is only a matter of time before bacteria will be released into the bloodstream, eventually leading to manifest infection and sepsis syndrome. Timely removal of a catheter may prevent subsequent bacteremic complications; it is impossible, however, to determine what this exact moment is for the individual patient. We postulate that a seeded infection – be it as a peripheral abscess or an infected thrombophlebitis – may continue to develop after the removal of the colonized CVC, initially even in the absence of clinical symptoms and SAB. The findings in **chapter VIIa and VIIb** support this: IV-catheter colonization with *S. aureus* is often complicated by SAB, even after removal of the catheter, and this complication may be prevented by prompt initiation of anti-staphylococcal therapy.

One may argue about what the exact mechanism is, and whether the absence of certain clinical parameters, combined with negative blood cultures drawn after removal of the catheter provide enough reassurance to forgo antibiotic treatment. A definitive answer to

this question would have to come from a prospective trial in which all intravascular-catheter tips are cultured, blood cultures are drawn at standardized moments and in which patients are randomized (double-blind) to either initiation of therapy at culture positivity or "standard treatment". Such a trial would, however, face severe practical diffulties. First of all a maximum of eight eligible patients per hospital per year are to be expected. Assuming an incidence of 10% subsequent bacteremia and a 80% reduction with standardized antibiotic treatment, the intervention and control arm would each need to contain 140 patients in order to achieve 80% power. If half the patients agree to study participation, it would take ten large hospitals seven years to – hopefully – include enough patients to finish the study. Secondly, since the IDSA-guidelines currently recommend treatment of all demonstrated cases of *S. aureus*-colonized intravascular catheters, a proposal for such a trial would probably not be warmly welcomed by medical ethical committees.

To cut a long story short: this study will never be performed. Standard treatment of all *S. aureus* colonized intravascular-catheter tips is the simplest and most effective option.

5.2 Management of colonized neurosurgical drains

The evidence supporting protocolized treatment of all CSF-drains colonized with *S. aureus* is – at least for the moment – less convincing than the evidence to treat all colonized IV-catheter tips. Drain-associated meningitis may cause severe morbidity in neurosurgical patients, but the course of the disease is not as rapidly progressive as *S. aureus* bacteremia, and although secondary meningitis is associated with a high mortality, attributable mortality appears low (**chapter V**).

Furthermore, drain-associated meningitis is in many respects a "soft" endpoint: positive cultures of CSF are not needed to establish an infection, and positive CSF cultures do not always prove infection. Strict criteria for pleiocytosis or biochemical markers are not available either, and clinical (i.e. neurological) symptoms are non-specific in many patients

who are at risk for drain-associated meningitis. In fact, according to the CDC-guidelines, the colonized drain itself may be considered a diagnostic criterion.

More reliable diagnostic criteria are urgently needed, since the treatment for secondary meningitis is both expensive and a burden on patients: it consists of two weeks intravenous (thus: hospitalized) antibiotic treatment. In practice, particularly the protocolized dismissal of the diagnosis and the cessation of antibiotic therapy when CSF cultures remain negative are not consistently followed. In **chapter VI**, an attempt was made to establish evidence-based criteria for the diagnosis of secondary meningitis. In our experience, the diagnosis often was not dismissed after negative cultures, due to perceived indications of infection, such as a WBC count > 10/mm^3 or WBC/RBC ratios > 1:500. Applying more strict criteria for the diagnosis secondary meningitis, we found higher cut-off points for WBC counts than generally applied, as well as quite variable WBC/RBC ratios. In practice, this means that a diagnosis of secondary meningitis may be rejected with more ease when CSF cultures remain negative and patient symptoms are non-specific or even absent.

6. Tertiary prevention: prevention of complications of CA-SAB

An infection which has already developed cannot be *prevented* anymore in the strictest sense of the word, but adequate management may limit complications and prevent attributable death: so-called tertiary prevention. Tertiary prevention relies on rapid identification of infections, timely initiation of therapy and immediate election of the optimal treatment modality. To put it in other words: “Speed is of the essence.”

In **chapter VIII** we attempted to quantify the contribution of the two main interventions in CA-SAB: initiation of antibiotic therapy and removal of the intravenous catheter. The goal was to establish an attributive mortality for each day these measures are delayed. The gathered data, however, did not permit to calculate such a number. In part, because the study was heavily biased in all directions of outcome. The results we found may have shown less mortality attributable to sepsis in patients whose antibiotic therapy was

initiated rapidly, but an influence on total all cause mortality was not measurable. A positive effect of rapid catheter removal could not be demonstrated. The main conclusion which could be drawn, is that in this study – as in all studies in which attempts are made to salvage the catheter – some patients whose IV-catheters remained in situ despite a firm diagnosis of CA-SAB succumbed to sepsis. These patient, thus, did not receive maximal treatment for their bacteremia.

Hesitation to remove an intravenous catheter causes, no doubt, attributable mortality, as does delay in initiation of antibiotic therapy, but to capture this effect in a "per day risk" proves challenging. Perhaps a large prospective cohort-study may answer this question, although the forcefully observational nature of such a study will always pose inherent difficulties in the interpretation of the results.

7. Keeping S. aureus infections manageable

A factor which adds to the complexity of managing HA-infections by *S. aureus*, is the bacterium's gradually increasing resistance to antibiotics. Since *S. aureus* is one of the foremost causes of sepsis, this increasing resistance affects the empiric therapy for nearly all infections. In settings with high MRSA-prevalence – approximately the entire world outside the Netherlands and Scandinavia – combinations with vancomycin, linezolid or daptomycin are necessary, and even these may not offer reliable protection. Resistance to vancomycin, until recently non-existent, is increasing, and basically there is not a single drug to which clinical resistance in *S. aureus* has *not* been reported.

Inevitably, at some point in the future the current antibiotic repertoire will not suffice, but few antibiotics are currently "in the pipeline", and no new antibiotic classes are. Considering the long and tedious road from active substance in the laboratory to an available, registered antibiotic, this is worrisome. To date, most antibiotics are (based on) inhibiting molecules produced by bacteria and fungi. It is not inconceivable that natural, microbiological sources will still yield novel leads and compounds. In **chapter IX** the

discovery, production and structural characterization of an antibiotic compound is detailed: epilancin 15X. This molecule exhibited activity against all *S. aureus* isolates tested, both MSSA and MRSA (MIC_{90} 4 mg/l), but systemic application will, for a number of reasons, not be feasible. However, many other similar molecules are being researched, and hopefully understanding of the mechanisms of action will ultimately lead to the development of viable therapeutic substances.

Future directions

A large part of this thesis deals with the difference between colonizing flora and pathogens. Basically, the questions are twofold. (1:) Is the outcome of a patient affected by the presence of a particular micro-organism on a particular body site / wound / medical device / prosthetic material? (2:) Will any intervention – either preventive, pre-emptive or therapeutic, and be it mechanical, surgical or pharmaceutical – improve this outcome?

This thesis was limited to certain types of infections and focused on *S. aureus*, but the underlying questions may be generalized to include other types of healthcare-associated infections and different micro-organisms. To name but a few:

"Is there any need to treat CR-BSI with coagulase-negative staphylococci or enterococci antibiotically once the infective focus, i.e. the IV-catheter has been removed?" Many clinicians believe there is not, but the IDSA-guidelines recommend 5-7 days (coagulase-negative staphylococci) or 7-14 days (enterococci) of antibiotic treatment, be it with level C-III recommendations (poor evidence based solely on expert opinion).

"What is the prognosis of bacteremia in patients with intravascular prosthetic materials?" Specifically: how probable is it that bacteremia reflects an infected vascular prosthesis, or that a prosthetic device becomes secondarily infected? Which risk factors are relevant? The micro-organism? The focus of infection? The type of prosthesis? The duration of bacteremia? The duration that a prosthesis has been in situ?

"Which micro-organisms in skin ulcerations influence the prognosis of the ulcer, and which should be treated?" We could look at decubitus ulcers, which are frequent complications of long-term hospitalization in malnourished patients, and which often progress into osteomyelitis. Cultures, even deep cultures of necrotic tissue, may yield *S. aureus* and Gram-negatives, but also regular skin flora. Is any benefit to be expected from antibiotic therapy over surgical management, and to which degree is this dependent on the isolated micro-organisms?

"How should wound infections be managed?" Is there a difference in effectiveness to be expected between local and systemic treatment of wound infections? And which signs and symptoms predict outcome and the necessity for antimicrobial treatment? Are some micro-organisms (e.g. *S .aureus* or *Pseudomonas aeruginosa*) always to be treated?

"What is the optimal duration of treatment for secondary meningitis?" Two weeks intravenous therapy is generally administered, but no evidence whatsoever exists on the matter. Would a week suffice for Gram-positive micro-organisms? Do these bacteria truly require treatment after a CSF-drain has been removed?

Answering any of these individual research questions with the highest level of evidence would require large, lengthy and costly randomized controlled multi-center trials. Even if considered of high enough priority to be allocated substantial funding, for many of the above questions the study-proposals would be unlikely to survive scrutiny by medical ethical evaluation boards, as they would conflict with established guidelines, leaving retrospective studies as the only tool to explore such subjects.

Although retrospective research is subject to all sorts of limitations in terms of data gathering, not to mention the unavoidable confounding resulting from observational study setups, it does also offer clear advantages. Apart from the relative speed with which retrospective studies may be performed, their inexpensive nature and the total absence of ethical impediments, the data gathered from them best approximate clinical reality. The quality of medical treatments is often hightened during prospective studies (a phenomenon known as the "Hawthorne effect"), which disturbs the validity of study outcomes for everyday patient care. In retrospective studies the instituted patient treatment is the everyday patient treatment, and effects may be observed as they occur in an actual hospital environment. In the end, this research could be regarded as the grouping of clinical experience transcending the individual medical doctor, with possibilities for a more systematic analysis of prognostic factors, and without relying on the capacity for recollection of our more experienced colleagues.

Currently hospital administration and patient history are progressively automated and recorded electronically. On the one hand, this improves the access to clinical data for retrospective research. On the other hand, in our limited experience, it appeared as if the electronic documentation by physicians did not equal the extensiveness of paper files. However, a larger threat to retrospective research comes from the restriction of access to patient data for reasons of patient privacy. Rules and legislation intended to guarantee confidentiality often construct psychological and practical hurdles for epidemiological research. Such hurdles are of course never prohibitive, but the added administrative workload hampers "quick and dirty" exploration of a subject – for instance to determine whether sufficient power may be achieved in a full study – and it may severely demotivate clinicians to initiate research. Had our hospital forced to request permission prior to every search in patient data, prior to even knowing whether a large enough patient group was available to answer a research question, the studies described in chapters IV through VIII of this thesis would likely never have been performed.

In conclusion: attempts to answer practical clinical questions can best first be made investigating the aggregated clinical data of a hospital's own patient files. Not only will this often yield higher levels of evidence than literature searches,[27] the findings will be truer reflections of current treatment modalities and diagnostic options in the local patient population, thus maximizing their validity for one's own institution.

References

1. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. **Am J Infect Control. 2008**; 36:309-32.

2. Klevens RM, Edwards JR, Richards CL, Horan TC, Gynes RP, et al. Estimating healthcare-associated infections and deaths in U.S. hospitals 2002. **Public Health Reports. 2007**; 122:160-166.

3. Vrijens F, Hulstaert F, Gords B, de Laet C, Devriese S, van de Sande S, et al. Nosocomiale infecties in België, deel 2: impact op mortaliteit en kosten. **KCE reports 2009**; 102A: www.kce.fgov.be.

4. de Bruijne MC, Zegers M, Hoonhout LH, Wagner C. Onbedoelde schade in Nederlandse ziekenhuizen. **2007**; www.onderzoekpatientveiligheid.nl/rapport.pdf

5. Ammerlaan HS, Herwaldt L, Hanberger H, Lingaas E, Harbarth S, Rohner P, et al. Trends in nosocomial MRSA bacteraemias: addition or replacement? The clinical impact of methicillin-resistant *Staphylococcus aureus* on morbitidy, mortality, and burden of disease. **2010**; p117-142.

6. Leavis HL, Bonten MJ, Willems RJ. Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. **Curr Opin Microbiol. 2006**; 9:454-460.

7. Oteo J, Pérez-Vázquez M, Campos J. Extended-spectrum β-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. **Curr Opin Infect Dis. 2010**; 23:320-6.

8. Lomaestro BM, Tobin EH, Shang W, Gootz T. The spread of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae to upstate New York. **Clin Infect Dis. 2006**; 43:e26-28.

9. Leverstein-van Hall MA, Blok HE, Paauw A, Fluit AC, Troelstra A, Mascini EM, et al. Extensive hospital-wide spread of a multidrug-resistant enterobacter cloacae clone, with late detection due to a variable antibiogram and frequent patient transfer. **J Clin Microbiol. 2006**; 44:518-524.

10. Stuart TL, Mulvey M, Simor AE, Tien HC, Battad A, Taylor G, et al. Acinetobacter baumannii in casualties returning from Afghanistan. **Can J Infect Control. 2007**; 22:152-154.

11. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. **N Engl J Med. 2010**; 362:9-17.

12. Wassenberg MW, de Wit GA, van Hout BA, Bonten MJ. Quantifying cost-effectiveness of controlling nosocomial spread of antibiotic-resistant bacteria: the case of MRSA. **PLoS One. 2010**; 5:e11562.

13. Xu G, Tu W, Xu C. Mupirocin for preventing exit-site infection and peritonitis in patients undergoing peritoneal dialysis. **Nephrol Dial Transplant. 2010**; 25:587-92.

14. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. **N Engl J Med. 2009**; 360:20-31.

15. Yilmaz G, Koksal I, Aydin K, Caylan R, Sucu N, Aksoy F. Risk factors of catheter-related bloodstream infections in parenteral nutrition catheterization. **JPEN J Parenter Enteral Nutr. 2007**; 31:284-287.

16. O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, et al. Guidelines for the prevention of intravascular catheter-related infections. Centers for Disease Control and Prevention. **MMWR Recomm Rep. 2002**; 51:1-29.

17. Lidwell OM, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Effect of ultraclean air in operating rooms on deep sepsis in the joint after total hip or knee replacement: a randomised study. **Br Med J (Clin Res Ed). 1982**; 285:10-14.

18. Kallen AJ, Patel PR, O'Grady NP. Preventing catheter-related bloodstream infections outside the intensive care unit: expanding prevention to new settings. **Clin Infect Dis. 2010**; 51:335-341.

19. Darouiche RO, Berger DH, Khardori N, Robertson CS, Wall MJ Jr, Metzler MH. et al. Comparison of antimicrobial impregnation with tunneling of long-term central venous catheters: a randomized controlled trial. **Ann Surg. 2005**; 242:193-200.

20. Halton KA, Cook DA, Whitby M, Paterson DL, Graves N. Cost effectiveness of antimicrobial catheters in the intensive care unit: addressing uncertainty in the decision. **Crit Care. 2009**; 13:R35.

21. McConnell SA, Gubbins PO, Anaissie EJ. Do antimicrobial-impregnated central venous catheters prevent catheter-related bloodstream infection? **Clin Infect Dis. 2003**; 37:65-72.

22. McConnell SA, Gubbins PO, Anaissie EJ. Are antimicrobial-impregnated catheters effective? Replace the water and grab your washcloth, because we have a baby to wash. **Clin Infect Dis. 2004**; 39:1829-1833.

23. Carson CC. Efficacy of antibiotic impregnation of inflatable penile prostheses in decreasing infection in original implants. **J Urol. 2004**;171:1611-1614.

24. Vester H, Wildemann B, Schmidmaier G, Stöckle U, Lucke M. Gentamycin delivered from a PDLLA coating of metallic implants In vivo and in vitro characterisation for local prophylaxis of implant-related osteomyelitis. **Injury. 2010**; Jun 11 (Epub ahead of print).

25. Agostinho A, James G, Wazni O, Citron M, Wilkoff BD. Inhibition of Staphylococcus aureus biofilms by a novel antibacterial envelope for use with implantable cardiac devices. **Clin Transl Sci. 2009**; 2:193-198

26. Schmidmaier G, Lucke M, Wildemann B, Haas NP, Raschke M. Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. **Injury. 2006**; 37:S105-12.

27. This thesis, chapters IV-VII.

XI

Nederlandse samenvatting & resumen en Español

El resumen en Español fue revisado por el doctor David Sevillano Fernández

Vele honderdduizenden miljarden bacteriën koloniseren elk menselijk lichaam. De meeste hiervan leveren ons geen ongemak, of zijn zelfs noodzakelijk voor ons functioneren, maar één in het bijzonder kan zich plotseling buitengewoon kwaadaardig ontpoppen: *Staphylococcus aureus*, letterlijk: "de gouden druiventros". Ongeveer één op de drie mensen draagt *Staphylococcus aureus* (*S. aureus*) met zich mee in de neus, en soms ook in de keel, in de darmen, op de huid, of in wonden en eczeem; doorgaans zonder er zelf weet van te hebben.

Een aantal eigenschappen van *S. aureus* maken hem uitzonderlijk gevaarlijk. In de eerste plaats beschikt de bacterie over allerhande moleculen waarmee hij zich goed kan hechten aan onze weefsels en aan medische materialen zoals infuuslijnen, kunstheupen en vaatprotheses. In de tweede plaats weet de bacterie, wanneer hij zich een plek heeft gevonden in ons lichaam, ons afweersysteem te omzeilen met een veelvoud aan trucs: hij kan signalen blokkeren die afweercellen moeten aantrekken, hij kan zich verbergen onder een kapsel of onder bloedstolsels, en hij kan de voor andere bacteriën dodelijke zuurstofradicalen zelf afbreken. Tenslotte produceert de bacterie een aantal gifstoffen: de lysines en toxines. Sommige gifstoffen vernietigen cellen, andere breken bindweefsel af, weer andere brengen het afweersysteem op hol (de zogenaamde "superantigenen").

Staphylococcus aureus is de voornaamste veroorzaker van ziekenhuisinfecties in het algemeen en van wondinfecties in het bijzonder. Het is ondoenlijk om vandaag de dag de impact hiervan op sterfte nog op waarde te schatten. Tijdens de Eerste Wereldoorlog waren wondinfecties verantwoordelijk voor meer dan de helft van de sterfte onder frontsoldaten. Ook veroorzaakte *S. aureus* waarschijnlijk ongeveer een kwart van de sterfgevallen tijdens de Spaanse griep van 1918. (De andere belangrijke doodsoorzaken waren infecties met pneumococcen, streptococcen en *Haemophilus influenza*.)

De ontdekking en commerciële productie van penicilline markeert dan ook een van de grootste doorbraken in de geneeskunde. Patiënten die voorheen na briljant geslaagde operaties overleden aan wondinfecties konden plotseling worden gered, en longontstekingen waren geen absoluut doodsvonnis meer. De overlevingskansen van gewonde soldaten

verbeterden dramatisch. Niet voor niets classificeerden tijdens de Tweede Wereldoorlog de Geallieerden het productieproces voor penicilline als militair geheim.

Net na de ontwikkeling van penicilline was *S. aureus* bijna universeel gevoelig voor het middel, maar al snel begonnen stammen te circuleren die er resistent tegen waren. Nieuwe antibiotica volgden: oxacilline, erythromycine, clindamycine, tetracycline, vancomycine, linezolid, daptomycine… maar bij elk middel vonden we na verloop van tijd stammen waartegen het niet werkte. De meeste problemen geven de *S. aureus* bacteriën die ongevoelig zijn voor de betalactamantibiotica (hieronder vallen bijvoorbeeld de penicillines): deze worden MRSA (methicilline-resistente *Staphylococcus aureus*) genoemd. De beta-lactamantibiotica zijn de antibiotica van eerste keuze voor staphylococcen, omdat andere middelen minder werkzaam zijn, meer bijwerkingen hebben, duurder, zijn of altijd per infuus moeten worden toegediend.

MRSA waart vooral in ziekenhuizen rond, zij het nauwelijks in Nederlandse ziekenhuizen. Dit is het resultaat van het strenge beleid dat hier geldt. Patiënten die risico lopen MRSA bij zich te dragen worden verpleegd met uitgebreide isolatiemaatregelen totdat vaststaat dat dit niet het geval is. Voorheen was het belangrijkste risico op dragerschap van MRSA een opname in een buitenlands ziekenhuis. Sinds 2004 is daar een nieuw risico bijgekomen: het werken met varkens of kalveren. Het is namelijk gebleken dat een zeer aanzienlijk deel van onze veestapel een bepaalde MRSA-bacterie, de “ST398-stam” of “varkens-MRSA”, bij zich draagt en deze regelmatig overdraagt aan haar verzorgers. Waarschijnlijk zijn deze bacteriën uitgeselecteerd door jarenlang gebruik van antibiotica in de veeteelt— antibiotica werken namelijk als groeibevorderaars op vee.

In **hoofdstuk 3** wordt een geval van een endocarditis (hartklepinfectie) met de varkens-MRSA beschreven. Dit geval is in de literatuur regelmatig aangehaald als bewijs dat de varkens-MRSA ernstige infecties kan veroorzaken. Het is echter de vraag in welke mate dit terecht is. Ondanks dat de patiënte ruim een week suboptimaal werd behandeld, en ondanks dat gewone MRSA-endocarditis meestal dodelijk verloopt, herstelde zij volledig. Daarnaast zijn, ondanks het inmiddels wijdverbreide voorkomen van de varkens-MRSA, nog steeds maar weinig ernstige infecties met deze bacterie beschreven.

Hoofdstuk 4 zocht naar bewijs voor de stelling dat *S. aureus* vanuit de bloedbaan in de urinewegen kan komen en dat een positieve urinekweek met deze bacterie een teken kan zijn van bloedbaaninfecties. Dit bewijs werd niet gevonden. Andersom juist, het leek erop dat de urinewegen een belangrijk ingangspunt waren voor infecties: meestal werd de bacterie al enkele dagen voordat de patiënten ziek werden gevonden in de urine. Urineweginfecties met *S. aureus* komen vaker voor dan voorheen aangenomen, maar gedragen zich meer als "wondinfecties van de urinewegen" dan als "gewone urineweginfecties". Aanwijzingen voor de urinewegen als ingangspunt voor *S. aureus* infecties elders in het lichaam zijn ook gevonden in latere studies. Met name wervelabcessen lijken hiermee verband te houden.

De moderne geneeskunde maakt veelvuldig gebruik van kunstmaterialen, een geliefd aangrijpingspunt van bacteriën. Sommige kunstmaterialen worden in steriele lichaamscompartimenten geplaatst: in de vorm van protheses of ingehechte matjes bijvoorbeeld. Andere kunstmaterialen zijn bedoeld als verbinding tussen de buitenwereld en het inwendige van de patiënt. De betekenis van bacteriële kolonisatie van deze laatste vorm werd in dit proefschrift tegen het licht gehouden. Er werd gekeken naar liquordrains en naar intraveneuze lijnen.

Liquordrains zijn plastic slangetjes ingebracht in een hersenkamer (een met vocht gevulde ruimte in het brein) of in de ruimte rond het ruggenmerg; ze laten de hersenvloeistof aflopen en om de hersendruk te verminderen. Het plaatsen van deze drains is de meest uitgevoerde neurochirurgische operatie. In **hoofdstuk 5** werd aangetoond dat groei van bacteriën op de liquordrains zelden onschuldig is. Patiënten op wier liquordrains bacteriën werden aangetoond hadden of kregen in de helft van de gevallen tevens een hersenvliesontsteking. *S. aureus* bleek de gevaarlijkste bacterie: drie kwart van de patiënten bij wie deze bacterie de drain koloniseerde had of kreeg een hersenvliesontsteking. **Hoofdstuk 6** beschreef hoe lastig het in de praktijk is om vast te stellen of een patiënt met een neurologische aandoening een hersenvliesontsteking heeft. Dit hoofdstuk geeft handvaten om de diagnose met meer zekerheid te kunnen stellen.

In **hoofdstukken 7 en 8** werd gekeken naar een van de belangrijkste gezondheidszorggerelateerde infecties: de lijninfectie met *S. aureus*. Intraveneuze lijnen of

"centrale lijnen" zijn catheters die zijn ingebracht in een grote ader of slagader. Ze worden in de gezondheidszorg gebruikt om medicijnen en bloedproducten toe te dienen, om minimaal-invasieve operaties uit te voeren, en om de bloeddruk en polsslag van patiënten te volgen. Bacteriën kunnen deze lijnen koloniseren en vervolgens in de bloedbaan komen. De reactie van het lichaam op een infectie in de bloedbaan, zogenaamde "sepsis" of "bloedvergiftiging", kan al dodelijk zijn, maar de bacterie kan zich hiervandaan ook verspreiden door het lichaam en aldaar abcessen veroorzaken.

Een van de vragen die we stelden was of het al voldoende reden is tot behandeling met antibiotica wanneer *S. aureus* wordt aangetoond op een centrale lijn. In **hoofdstuk 7** keken we in twee afzonderlijke studies naar de prognose van patiënten met gekoloniseerde lijnen. Het bleek dat bijna de helft van hen op het moment dat de lijn werd uitgenomen al positieve bloedkweken had; van de andere helft kreeg één op de zeven positieve bloedkweken na het uitnemen van de lijn. Behandeling met antibiotica beschermde tegen complicaties met *S. aureus*: hoewel de patiënten die antibiotica kregen doorgaans zieker waren, traden er meer complicaties op bij de groep patiënten die géén antibiotica kreeg; deze complicaties varieerden van bot- en gewrichtsinfecties tot zelfs sterfgevallen door bloedvergiftiging.

Onder andere op basis van deze studies zijn in 2009 de richtlijnen van de Amerikaanse Vereniging voor Infectieziekten (IDSA) aangepast. In de nieuwe richtlijn voor de diagnostiek en behandeling van lijninfecties wordt geadviseerd standaard vijf tot zeven dagen antibiotica te geven wanneer een intraveneuze lijn is gekoloniseerd met *S. aureus*.

Hoofdstuk 8 gaat in op de moeilijkheden om behandeleffecten aan te tonen bij patiënten met bloedvergiftiging door lijninfecties met *S. aureus*. We weten dat als je niet op tijd goed behandelt bijna alle patiënten met deze infectie zullen overlijden; wat we niet weten, is wat "op tijd" precies inhoudt en wat geldt als "goede behandeling". Het lijkt erop dat een dag eerder beginnen met antibiotica in een gedeelte van de patiënten levensreddend kan zijn, maar omdat veel van de patiënten die deze infectie oplopen van tevoren al heel ernstig ziek zijn, vaak zelfs dodelijk ziek, is het erg moeilijk hier goed onderzoek naar te doen.

De immer toenemende resistentie van *S. aureus* tegen antibiotica kan in grote delen van de wereld niet meer bestreden worden met louter isolatiemaatregelen en instelling van een meer verantwoord antibioticagebruik; nieuwe antibiotica zullen moeten worden ontwikkeld. **Hoofdstuk 9** beschrijft een aantal eerste stappen van een proces dat uiteindelijk hopelijk aanknopingspunten ("leads") zal geven voor de ontwikkeling van nieuwe antibiotica. De stof die hier gevonden, geïsoleerd en getypeerd werd, "epilancine 15X", is onder andere actief tegen MRSA. Hoewel het onwaarschijnlijk is dat epilancine 15X ooit zal worden ingezet als therapeutisch middel in mensen, kan analyse van het werkingsmechanisme wellicht op een dag een bruikbaar antibioticum opleveren.

Voor dit idee geldt echter wat voor zo vele stellingen, theorieën en hypothesen in de geneeskunde geldt:

…nader onderzoek zal het moeten uitwijzen.

Cientos de miles de billones de bacterias pueblan el cuerpo humano. La mayoría son colonizadores inofensivos de mucosas o desempeñan un papel indispensable para el funcionamiento de nuestros organismos. Comparativamente hablando, el porcentaje de la población bacteriana colonizadora que demuestra algún tipo de virulencia hacia el ser humano, es insignificante. Pero es precisamente este pequeño número de bacterias el que, por motivos obvios, nos interesa desde un punto de vista médico. El *Staphylococcus aureus* (*S. aureus*), literalmente "racimo de uvas de oro", es una especie patógena para el ser humano que se manifiesta de un modo extremamente malicioso. Aproximadamente, uno de cada cuatro humanos porta el microorganismo en nariz, y con menor frecuencia en faringe, intestino, en lesiones y eccemas, en general sin ocasionar patología alguna. Sin embargo el *S. aureus* presenta un número de propiedades, o factores de virulencia, que lo convierten en un microorganismo potencialmente invasivo. La bacteria dispone de un arsenal de moléculas que le permiten adherirse a nuestros tejidos y materiales médicos, vías de acceso venoso, prótesis articulares o vasculares. Una vez establecida en el organismo, la bacteria dispone de múltiples estrategias, útiles para eludir al sistema inmune. Es capaz de bloquear las señales que atraen a las células inmunitarias, de ocultarse debajo de su cápside o de coágulos, y de desactivar los radicales libres producidos por nuestros glóbulos blancos (letales para otras bacterias). Además es un microorganismo productor de diversas toxinas con capacidad para destruir células, tejidos o estimular, literalmente enloquecer, al sistema inmune (los "superantigenos").

El *S. aureus e*s la principal causa de infecciones hospitalarias, en general, y de infecciones de heridas en particular. Durante la Primera Guerra Mundial, las infecciones de heridas por *S. aureus* ocasionaron más de la mitad de las muertes de los soldados en el frente. Es probable que el *S. aureus* fuese también el agente responsable de una cuarta parte de las muertes ocurridas durante la epidemia de "la gripe española" del 1918 (otras causas fueron infecciones por neumococos, estreptococos o *Haemophilus influenzae*.)

El descubrimiento y producción de la penicilina a gran escala, marca uno de los mayores hitos en la historia de la medicina. De repente podían ser salvados pacientes que

poco tiempo antes, después de ser sometidos a brillantes operaciones, fallecían por infecciones en las heridas. Las neumonías dejaron de ser una sentencia de muerte. Las posibilidades de supervivencia de los soldados heridos en combate aumentaron drásticamente, por lo que, no es de extrañar que los propios Aliados durante la Segunda Guerra Mundial clasificaran el proceso de producción de la penicilina como un secreto militar.

En un inicio, las cepas de *S. aureus* eran completamente sensibles a la penicilina. Sin embargo pronto comenzaron a seleccionarse variantes o cepas resistentes que más tarde se diseminaron en el ambiente. El desarrollo de nuevos antimicrobianos, oxacilina, eritromicina, clindamicina, tetraciclina, vancomicina, incluso linezolid o daptomicina, lejos de eliminar el problema (aunque son indiscutibles sus beneficios) han proporcionado, con una mayor o menor implicación, la selección de cepas multirresistentes. Un subgrupo ampliamente extendido de cepas resistentes de *S. aureus*, es el que presenta resistencia a los antibióticos betalacámicos, el "SARM" o *S. aureus* resistente a meticilina (la meticilina aquí se considera el representante de todos los betalactámicos). Los betalactámicos son los antimicrobianos de preferencia para el tratamiento de las infecciones relacionadas con el *S. aureus*, por actividad, menor frecuencia de aparición de efectos adversos, posibilidad de administración por vía oral o intravenosa o mejor perfil farmacoeconómico que otros antibacterianos.

El aislamiento del SARM es un problema frecuente y extendido a los hospitales de todo el mundo. Sin embargo en Holanda se trata de un problema aparente (la prevalencia del SARM continúa siendo muy baja) resultado de las estrictas normas de prevención y control autoimpuestas. Todos los pacientes con riesgo de portar SARM reciben, hasta que sea confirmada la ausencia del microorganismo, medidas preventivas encaminadas a limitar la posible diseminación de la bacteria. Hasta hace relativamente poco tiempo el principal factor de riesgo de colonización por SARM era el ingreso hospitalario en un centro sanitario extranjero. Sin embargo en el año 2004 se introdujo como factor de riesgo potencial el manejo de ganado porcino o vacuno, como consecuencia de la diseminación entre nuestra cabaña ganadera de una variante de SARM, la cepa "ST-398" o "cepa porcina", que con

regularidad era transmitida a los ganaderos. Es posible, aunque solo son especulaciones, que esta variante fuera seleccionada por el abuso de antimicrobianos en ganadería, conocidos estimulantes del crecimiento de los animales de granja.

En el **capítulo 3** se describe un caso clínico de endocarditis (infección asociada a válvula cardiaca) asociado a la cepa porcina del SARM. Desde su publicación el caso clínico ha sido referido con relativa frecuencia en la literatura científica como prueba fehaciente de la capacidad de la variante porcina de SARM para causar infecciones graves. Sin embargo, desde nuestro punto de vista se trata de un aspecto discutible en base a que (i) la paciente infectada se recuperó por completo aun habiendo recibido dosis subterapeúticas durante una semana de tratamiento para un proceso (las endocarditis por SARM) que por lo general suele ser mortal y (ii) que las infecciones descritas por esta cepa son muy escasas para la extensa diseminación en el ambiente que se le presume.

En el Capítulo 4 refutamos la hipótesis popularmente aceptada que establece que las infecciones de *S. aureus* en vías urinarias (bacteriuria) son consecuencia de la presencia de una infección de la sangre (bacteriemia). De esta forma, el aislamiento de *S. aureus* en orina es indicativo de la existencia de la bacteriemia. Sin embargo, de acuerdo a nuestros resultados, el sistema urinario sería una importante vía de acceso del microorganismo y la frecuencia de bacteriurias por *S. aureus*, infecciones que comparten más puntos en común con las de heridas que con las cistitis comunes, se estima superior a la descrita en la actualidad. En un amplio número de casos se encontró que es realmente la presencia del microorganismo en orina la que precede a la aparición de la bacteriemia. Estudios posteriores han revelado que la vía urinaria es también un punto de entrada para otras infecciones de *S. aureus* como los abscesos vertebrales.

Los dispositivos artificiales, como prótesis, drenajes o catéteres de acceso venoso, son un recurso terapéutico de uso extendido en la medicina moderna que frecuentemente sitúa al paciente en una situación de riesgo para el desarrollo de infecciones. Por este motivo, los siguientes capítulos están dedicados a conocer el significado de la colonización bacteriana de dos tipos de dispositivos de uso habitual como los drenajes de líquido cefalorraquídeo y los catéteres de acceso venoso. Un drenaje de líquido cefalorraquídeo (de

LCR) es un tubo de plástico insertado en un ventrículo cerebral (estructura anatómica del cerebro que conforma el sistema ventricular por el que circula líquido cefalorraquídeo) o próximo a la médula espinal a través del que se libera LCR superfluo con el objetivo de bajar la presión en el cerebro. La inserción de drenajes de LCR es hoy por hoy el proceso neuroquirúrgico más frecuentemente realizado. En el **capítulo 5** se demuestra que el aislamiento de bacterias en los drenajes de LCR se encuentra fuertemente asociado con el desarrollo de complicaciones secundarias. La mitad de los pacientes con cultivos positivos en los drenajes desarrollan meningitis secundaria, que aumenta a tres de cada cuatro pacientes cuando el microorganismo colonizador es el S. *aureus*. El **Capítulo 6** describe las dificultades prácticas existentes a la hora de determinar si un paciente con enfermedad neurológica (en general con un infarto cerebral) padece o no una meningitis bacteriana y se sugieren estrategias para mejorar su diagnóstico.

En los **Capitulos 7 y 8** se explora una de las infecciones sanitarias más actuales, la infección de catéteres intravenosos asociada a *S. aureus*. Los catéteres venosos, o "líneas venosas centrales" son dispositivos insertados en cualquiera de los vasos mayores (venas o artérias), usados para administrar al paciente fluidos, derivados sanguíneos, fármacos, llevar a cabo operaciones mínimamente invasivas, monitorizar el pulso o la tensión arterial. La colonización de estos dispositivos origina infecciones en la sangre "sepsis o septicemia" y disemina el microorganismo por el organismo ocasionando abscesos. El pronóstico es por lo general grave, con riesgo para la integrad del paciente.

En el **Capítulo 7** se describe, en dos estudios separados, la prognosis de los pacientes que presentan líneas centrales colonizadas por el microorganismo, con el objetivo de discernir si el cultivo de *S. aureus* en una línea central es razón suficiente para iniciar el tratamiento antimicrobiano. De acuerdo con nuestros resultados, casi la mitad de los pacientes incluidos en el estudio presenta cultivos positivos en sangre en el momento de extraer la línea central y uno de cada siete pacientes con cultivos negativos desarrolla bacteriemia posterior a la extracción. El tratamiento con antibacterianos parece reducir el riesgo de complicaciones por *S. aureus*, que en general varían desde artritis y osteomielitis hasta el fallecimiento por septicemia. Los resultados obtenidos en este trabajo han sido

utilizados por la Asociación Americana para Enfermedades Infecciosas (IDSA) durante la actualización de las recomendaciones para el manejo de las infecciones asociadas a catéteres. El nuevo documento de la IDSA ya recoge la necesidad de administrar un tratamiento antimicrobiano durante un periodo de cinco a siete días cuando una línea venosa se encuentra colonizada por *S. aureus*.

En el **Capítulo 8** se describe como la administración precoz de un tratamiento puede modificar la prognosis de los pacientes con septicemia por *S. aureus* asociada a línea venosa central. El objetivo de este capítulo se centró en determinar cuánto tiempo debe anticiparse el tratamiento para improvisar cierta supervivencia a los pacientes infectados. Según nuestros resultados comenzar el tratamiento con un día de antelación aumenta el éxito clínico en algunos grupos de riesgo. Sin embargo las enfermedades de base preexistentes en los pacientes analizados no permiten extraer firmes conclusiones.

La diseminación de cepas multiresistentes de *S. aureus* alrededor del mundo obliga a adoptar estrategias drásticas para su control. El aislamiento de los pacientes infectados o pacientes con riesgo de infección o la restricción del uso de antimicrobianos, son algunas de las medidas tipo adoptadas de forma habitual. Sin embargo, los escasos recursos terapéuticos disponibles frente a estas cepas resistentes hacen que la búsqueda de nuevas moléculas activas sea de vital importancia. En el **Capítulo 9** se describen las primeras fases de desarrollo de un posible candidato. La molécula propuesta en este capítulo, la epilancina 15X, una vez aislada y caracterizada demuestra tener un espectro de actividad que incluye al SARM. Es probable que la epilencina 15X nunca tenga uso en humanos, pero su mecanismo de acción puede servir de modelo para el diseño de moléculas con un uso comercial. Como en otros casos, teorías e hipótesis que llenan nuestros libros de medicina, la utilidad de la epilencina 15X…

…deberá ser demonstrada en futuros experimentos.

XII

Acknowledgements, Curriculum Vitae,
Healthcare-associated publications

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Curriculum vitae

Miquel Bart Ekkelenkamp Bulnes was born on the 6th of July 1976 in Bloomington, Indiana (United States of America). He grew up in Zoetermeer and Ugchelen and graduated from the Gymnasium Apeldoorn in 1994. That same year he started his medicine study at the University of Utrecht. During this study he spent half a year doing molecular research on Japanese encephalitis virus at the laboratory of Dr Alan Barrett at the University of Texas, Medical Branch on the island of Galveston.

After graduating in medicine on January 26th 2001, he first worked a year as a resident in internal medicine, pneumology and cardiology at the Groene Hart Hospital in Gouda. In 2002 he started both the research on this thesis and his residency in clinical microbiology at the Utrecht University Medical Center, under the supervision of prof. dr. Jan Verhoef and dr. Annemarie Weersink. Part of the residency took place at the Diakonessen Hospital in Utrecht, under supervision of dr. Rob Diepersloot. Once the training was finished on September 1st 2007, he took a sabbatical year to travel and write.

Since November 2008 he works as a clinical microbiologist at the Utrecht University Medical Center (head of the department: prof. dr. Marc Bonten). Furthermore, he regularly publishes novels, columns and opinions.

Healthcare-associated publications

Hetem DJ, de Ruiter SC, Buiting AG, Kluytmans JA, Thijsen SF, Vlaminckx BJ, Wintermans RG, Bonten MJ, Ekkelenkamp MB. *Staphylococcus aureus* bacteremia and sepsis is preventable in patients with *Staphylococcus aureus* colonization of intravascular catheters. **Medicine (Baltimore)**. Accepted.

Mooiweer E, Luijk B, Bonten MJ, Ekkelenkamp MB. C-reactive protein levels but not CRP dynamics predict mortality in patients with pneumococcal pneumonia. **J Infection**. 2011; February 4th.

Ekkelenkamp MB, Bonten MJ. Dual pneumococcal and influenza vaccination: severe concerns about the composition of the control groups. (Letter.) **Clin Infect Dis**. 2011; 52:.557-558.

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; 113:1309-1313.

Kanis MJ, Oosterheert JJ, Lin S, Boel CH, Ekkelenkamp MB. Corneal graft rejection complicated by *Paracoccus yeei* infection in a patient who had undergone a penetrating keratoplasty. **J Clin Microbiol**. 2010; 48:323-325.

Ekkelenkamp MB, Rooijakkers SH, Bonten MJ. Staphylococci and micrococci. In: *Infectious Diseases*, 3rd edition. Editors: Cohen J, Opal S, Powderly WG. 2010.

Ekkelenkamp MB. "Experts" fed the panic on television and now we are stuck with far too many vaccines. **NRC Handelsblad**. 2009; September 28

Ekkelenkamp MB. The Mexican flu is being hyped. **NRC Handelsblad**. 2009; May 9

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: 114-118.

Ekkelenkamp MB, Verhoef J, Bonten MJ. Quantifying the relationship between *Staphylococcus aureus* bacteremia and *S. aureus* bacteriuria: a retrospective analysis in a tertiary care hospital. **Clin Infect Dis**. 2007; 44:1457-1459.

Ekkelenkamp MB, Sekkat M, Carpaij N, Troelstra A, Bonten MJ. Endocarditis due to meticillin-resistant *Staphylococcus aureus* originating from pigs. **Ned Tijdschr Geneeskd**. 2006; 150:2442-2447.

Ekkelenkamp MB, Bonten MJ. Colistin as last resort in 2 patients with pulmonary infection with multiresistant nosocomial bacteria. **Ned Tijdschr Geneeskd**. 2006; 150: 1022-1026.

Ekkelenkamp MB, Hanssen M, Danny Hsu ST, de Jong A, Milatovic D, Verhoef J, van Nuland NA. Isolation and structural characterization of epilancin 15X, a novel lantibiotic from a clinical strain of *Staphylococcus epidermidis*. **FEBS Lett**. 2005; 579:1917-1922.

Ekkelenkamp MB, de Jong W, Hustinx W, Thijsen SF. *Streptomyces thermovulgaris* bacteremia in Crohn's disease patient. **Emerg Infect Dis**. 2004; 10:1883-1885.

Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ, Barrett AD. Origin and evolution of Japanese encephalitis virus in southeast **Asia. J Virol**. 2003; 77:3091-3098.

Janssen R, Weda M, Ekkelenkamp MB, Lammers JW, Zanen P. Metal versus plastic spacers: an in vitro and in vivo comparison. **Int J Pharm**. 2002; 245:93-98.

Robb CW, Orihuela CJ, Ekkelenkamp MB, Niesel DW. Identification and characterization of an in vivo regulated D15/Oma87 homologue in *Shigella flexneri* using differential display polymerase chain reaction. **Gene**. 2001; 262:169-177.