Epidemiology,
Treatment and Outcome of
Staphylococcus aureus
Bacteremia and Endocarditis

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three original publications and one manuscript submitted for publication, referred to in the text by their Roman numerals I-IV.

I   Lyytikäinen O, Ruotsalainen E, Järvinen A, Valtonen V, Ruutu P. Trends and outcome of nosocomial and community-acquired bloodstream infections due to *Staphylococcus aureus* in Finland, 1995-2001. *Eur J Clin Microbiol Infect Dis* 2005; 24:399-404.

II   Ruotsalainen E, Järvinen A, Koivula I, Kauma H, Rintala E, Lumio J, Kotilainen P, Vaara M, Nikoskelainen J, Valtonen V. Levofloxacin does not decrease mortality in *Staphylococcus aureus* bacteraemia when added to the standard treatment: A prospective and randomized clinical trial of 381 patients. *J Intern Med* 2006; 259:179-190.

III   Ruotsalainen E, Sammalkorpi K, Laine J, Huotari K, Sarna S, Valtonen V, Järvinen A. Clinical manifestations and outcome in *Staphylococcus aureus* endocarditis among injection drug users and nonaddicts: A prospective study of 74 patients. *BMC Infect Dis* 2006; 6:137.

IV   Ruotsalainen E, Karden-Lilja M, Kuusela P, Vuopio-Varkila J, Virolainen-Julkunen A, Sarna S, Valtonen V, Järvinen A. *Staphylococcus aureus* bacteremia and endocarditis among injection drug users and nonaddicts: Host factors, microbiological and serological characteristics. Submitted.

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
APACHE II	acute physiology and chronic health evaluation
ARDS	adult respiratory distress syndrome
ASTA	antibody against staphylolysin or staphylococcal α-haemolysin
bid	twice daily
CI	confidence interval
CMH	Cochran-Mantel-Haenszel test
CNS	central nervous system
CRP	C-reactive protein
DIC	disseminated intravascular coagulation
HILMO	national hospital discharge registry
HIV	human immunodeficiency virus
HR	hazard ratio
ICU	intensive care unit
IDUs	injection drug users
IE	infective endocarditis
IQR	interquartile range
ITT	intention-to-treat analysis
IU/ml	international units per milliliter
MIC_{90}	minimum concentration required to inhibit 90% of isolates
MLST	multilocus sequencing typing
MSCRAMMs	microbial surface components recognizing adhesive matrix molecules
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
NIDR	National Infectious Diseases Register
OR	odds ratio
PFGE	pulsed-field gel electrophoresis
PP	per-protocol analysis
PVL	Panton-Valentine leukocidin
RMANOVA	Analysis of Variance for Repeated measurements
SAB	*Staphylococcus aureus* bacteremia(s)
SAK	staphylokinase
SCC*mec*	staphylococcal cassette chromosome *mec*
TAA	antibody against teichoic acid
TEE	transesophageal echocardiography
TTE	transthoracic echocardiography
vs.	versus
wk	week

ABSTRACT

Introduction. *Staphylococcus aureus* is the second most common bloodstream isolate both in hospital- and community-acquired bacteremias and it still confers remarkably high mortality, especially in endocarditis. The clinical course of *S. aureus* bacteremia (SAB) is determined by its complications, particularly by the development of deep infections due to metastatic spread and thromboembolic events. However, injection drug users (IDUs) tend to have fewer complications and better prognosis than nonaddicts. The present studies were undertaken to investigate I) trends in the incidence, outcome and morbidity of SAB in Finland during 1995-2001, II) whether levofloxacin added to standard treatment of SAB would improve the patient outcome such as reduced mortality or complications, III) differences in clinical manifestations and outcome of *S. aureus* endocarditis among IDUs and nonaddicts, and IV) bacterial strains and their virulence factors, and host immune responses in order to explain the different prognosis and risk of developing endocarditis among IDUs and nonaddicts.

Subjects and methods. Study I was a retrospective epidemiological population-based study, in which trends in the age- and sex-specific annual incidence, proportion of nosocomial versus community-acquired bacteremia, and outcome during 1995-2001 were evaluated in 5045 SAB cases. In Study II, 381 consecutive patients with SAB were randomized into two groups receiving either standard antibiotic treatment or levofloxacin added to standard treatment. Primary end-points were mortality at 28 days and at three months. Clinical and laboratory parameters were analyzed as secondary end-points. The study made a *post hoc* analysis on the effect of rifampicin on mortality among patients with a deep infection available. Studies III and IV consisted of 430 SAB patients followed prospectively for three months. In Study III, all 74 patients with endocarditis were included of whom 20 were IDUs and 54 nonaddicts. Mortality and clinical parameters were compared between these groups. In Study IV, all 44 IDUs were identified and 20 of them had endocarditis. An equal number of nonaddicts were chosen as group matched controls. *S. aureus* isolates were genotyped by pulsed-field gel electrophoresis, and tested for Panton-Valentine leukocidin, staphylokinase, protease, and haemolysin production. Acute and convalescent sera were tested for antibodies to α-haemolysin (ASTA) and teichoic acid (TAA).

Results. The annual incidence of SAB in Finland increased significantly from 11 to 17 cases per 100,000 population during 1995-2001, most distinctly in elderly persons. Nosocomial infections accounted for 51% of cases, with no change in their proportion. The 28-day mortality was 17%, and did not change over time. Mortality increased with age, being highest among persons

aged >74 years. The risk of death at seven and 28 days, and at three months was significantly higher among nosocomial cases than among community-acquired cases. Additional levofloxacin treatment in SAB did not decrease mortality or the incidence of deep infections, nor did it speed up recovery. Deep infection was found in 84% of SAB patients already within one week after randomization. Interestingly, mortality for patients with deep infection was significantly lower among those who received rifampicin as compared to those treated without rifampicin (17% vs. 38%). Endocarditis was more frequently connected to SAB in IDUs. Right-sided endocarditis predominated among addicts whereas most of the nonaddicts had left-sided involvement. However, IDUs had equally often extracardiac deep infections (85% vs. 89%), arterial thromboembolic events (25% vs. 32%), and severe sepsis (45% vs. 52%) as nonaddicts. Injection drug abuse in accordance with younger age and lack of underlying diseases were associated with lower mortality. Only a small proportion of patients developed any serological response in SAB with a deep infection. Interestingly, the initial ASTA titer was more often positive among IDUs without endocarditis than those with endocarditis. No individual strain was associated with endocarditis among addicts. Characterization of the virulence factors of strains did not reveal any differences in IDUs and nonaddicts. However, haemolytic properties were found more often among IDUs without endocarditis than with endocarditis.

Conclusions. *Staphylococcus aureus* bloodstream infections increased in Finland during 1995-2001. While the increasing in incidence may partly be due to increased reporting, it also reflects a growing population at risk, affected by such factors as high age and/or severe comorbidity. However, the outcome of SAB remained unchanged during the seven years period and at a lower level than previously reported worldwide. Interestingly, the changes we detected in the epidemiology of SAB overall are very similar to those found in countries with a high prevalence of methicillin-resistant *S. aureus*. The trial reported here on levofloxacin treatment comprises one of the largest prospectively followed SAB populations worldwide. Deep infections were found in more patients than previously reported suggesting that they should be intensively searched for in SAB. Although levofloxacin added to standard treatment did not improve outcome, we found that patients with deep infection had lower mortality when treated with rifampicin combination therapy. In contrast to earlier reports in endocarditis, IDUs showed extracardiac deep infections and thromboembolic events with the same high frequency as nonaddicts. In spite of this, mortality was significantly lower among IDUs in agreement with previous data. The studied host immune responses and pathogen characteristics did not seem to explain the difference in clinical outcome between IDUs and nonaddicts. Furthermore, serological tests were not helpful in identifying patients with a deep infection.

1. INTRODUCTION

Staphylococcus aureus is one of the most important pathogens in community- and hospital-acquired bacteremias in all age groups. *S. aureus* bacteremia (SAB) is presently ranked second in Finland with approximately 1000 cases annually,[1,2] and the prevalence of bacteremic methicillin-resistant *S. aureus* (MRSA) infections is less than 3%.[2] The clinical course of SAB is determined by its complications, particularly by the development of deep infections due to metastatic spread, and by thromboembolic events and the high recurrence rate of bacteremia.[1,3-7] Furthermore, *S. aureus* has emerged as a leading cause of endocarditis and is observed in 11% to 35% of patients with SAB.[5-7] Classic risk factors for endocarditis such as rheumatic heart disease are being replaced by new ones, including the increased incidence of injection drug users (IDUs), the elderly with degenerative valve disease, patients with an intravascular catheter or a prosthetic valve, and nosocomial acquisition.[1,4,8]

Despite the progress of antimicrobial therapy, bacteremia due to *S. aureus* is still associated with mortality ranging from 7% to 39% in recent publications.[3,6,9-14] The factors associated with a poor prognosis include, in particular, MRSA strains, non-removable infection foci, metastatic foci (i.e., deep infections), thromboembolic events and central nervous system manifestations. IDUs tend to have a lower mortality rate (from 2% to 12%) than general population.[19-21] The better prognosis among IDUs is generally thought to be explained by host factors such as younger age, lack of valvular or other underlying diseases, and right-sided involvement in endocarditis.

Bacteremic *S. aureus* infections are recommended to be treated with intravenous antibiotic therapy, which should continue for several weeks in endocarditis or in other deep infections.[22,23] The standard treatment has been based on beta-lactam antibiotics in countries with a low prevalence of methicillin resistance. Newer antistaphylococcal agents such as linezolid, daptomycin, or quinupristin-dalfopristin have been used in infections caused by MRSA strains or strains with reduced susceptibility to vancomycin but no dramatic superiority in comparison to the standard therapies was observed in most of the clinical trials.[24-26] Combination therapy with an aminoglycoside and a beta-lactam is recommended in endocarditis.[27,28] According to experimental studies and some small clinical trials, rifampicin added to standard therapy in serious staphylococcal infections has been observed to be more effective than single drug therapy.[29-31] However, the clinical use of rifampicin has remained controversial and it is recommended variably

in deep-seated abscesses, osteomyelitis, prosthetic valve endocarditis and other foreign body infections, or because of a poor response to the standard treatment.[22,28,32-35]

New fluoroquinolones with improved activity against gram-positive bacteria have been introduced. Levofloxacin, trovafloxacin and moxifloxacin exhibit activity against methicillin-sensitive *S. aureus* with the MIC_{90} ranging from 0.06 mg/L to 0.5 mg/L.[36,37] In experimental studies they have shown an additive effect in combination with semisynthetic penicillin.[38] In SAB, most deep infections are evident within two weeks after the onset of bacteremia.[16,39] These metastatic infections might be prevented by early treatment with a bactericidal fluoroquinolone, which penetrates well into tissues. The efficacy of a fluoroquinolone added to beta-lactam therapy in SAB has not been evaluated in clinical trials.

The present studies were undertaken to gain information on epidemiology, treatment and outcome of SAB. We evaluated retrospectively the trends in the incidence and outcome due to *S. aureus* bloodstream infections in Finland during 1995-2001. We also conducted a prospective and randomized multicenter study to find out if newer fluoroquinolones (trovafloxacin or levofloxacin) combined with the present antistaphylococcal treatment of SAB would improve the prognosis and reduce complications such as deep infections and thromboembolic events. In addition, we evaluated risk factors, differences in clinical manifestations and outcome among IDUs and nonaddicts in *S. aureus* endocarditis. Finally, we investigated if the bacterial strains and their virulence factors, and host immune responses could explain the different prognosis and risk of developing endocarditis among IDUs as compared to nonaddicts.

2. REVIEW OF THE LITERATURE

2.1. Epidemiology of invasive *Staphylococcus aureus* infections

2.1.1. Incidence of *Staphylococcus aureus* bacteremia and endocarditis

S. aureus is one of the most frequently isolated pathogens in blood cultures in all age groups and it accounts for 19% to 25% of all bloodstream infections worldwide.[40-43] *S. aureus* bacteremia (SAB) ranks second in Finland with approximately 1000 cases annually, and is caused predominantly by methicillin-sensitive strains.[1,2] The epidemiology of bloodstream infections due to *S. aureus* has been studied mostly in selected hospitals which may not be representative of all healthcare facilities.[7-9,44-47] In addition, only a few population-based studies, which identify trends in the incidence and outcome of the disease over time and would allow comparisons between countries, have been published previously.[10,48,49] The age- and sex-specific incidence rates have rarely been reported.[48,50-52] Furthermore, most current population-based studies have focused on the contribution of methicillin resistance to morbidity and mortality associated with SAB.[15,49,53,54]

A recently published nationwide population-based survey in Denmark, a country with a very low prevalence of methicillin-resistant *S. aureus* (MRSA), reported that the incidence of SAB during 1971-2000 had increased two-fold from 14 to 31 cases per 100,000 population, representing an annualized increase of 4%.[10] The overall SAB incidence rates in the United States and Australia were of a similar magnitude or slightly higher than in the preceding survey[47,52] but in Canada, Wales and Northern Ireland the rates were much lower (from 10 to 20 cases per 100,000 population).[49,51,53,54] Furthermore, the major increase in incidence has occurred in males and in age groups <1 year and >65 years old.[10,48-52,55,56]

The incidence of infective endocarditis (IE) has not changed during the past two decades.[22,57] However, the historical predominance of streptococcal endocarditis is being replaced by *S. aureus* valve infection in many regions of the world.[21,58-60] A recently published population-based study reported that the adjusted annual incidence of *S. aureus* endocarditis was one to two cases per 100,000 population.[61] The increased frequency of *S. aureus* endocarditis is primarily a consequence of an increased use of invasive procedures, steadily rising rates of nosocomial bacteremia and injection drug abuse, and improvements in diagnostic techniques.[21,22,57,58,62-64] During the recent decades endocarditis has been observed in 11% to 35% of patients with SAB,[65-68] and more frequently among injection drug users (IDUs) in 35% to 67% of cases.[65,69-72]

Advances in echocardiography and the use of validated diagnostic criteria for IE have also contributed to the incidence of endocarditis in SAB patients.[73-76]

2.1.2. Community- and hospital-acquired bacteremia

Frequencies of both community- and hospital-acquired SAB have been increasing steadily[44,77,78] although SAB has been predominantly a nosocomial infection over the decades.[50,52,79-81] Patients with community-onset bacteremia are younger, and have more often unknown infection foci and metastatic infections (e.g., endocarditis or osteomyelitis), probably due to a longer duration of bacteremia before diagnosis and treatment when compared to patients with nosocomial SAB.[17,82-85] Patients with hospital-acquired SAB are older and the source of bacteremia is known in nearly all patients.[17,84] Nosocomial bacteremia is mostly associated with the expanding use of invasive procedures, and the presence of prosthetic devices, and intravascular or urinary catheters.[79,86] Because of the increased use of long-term intravascular devices in patients with chronic diseases in non-hospital settings, a new patient group of catheter-related community-onset bacteremia has emerged.[44,76,78]

2.1.3. Methicillin-resistant *Staphylococcus aureus*

The global emergence of drug-resistant bacteria is a pressing public health problem. Methicillin resistance in *S. aureus* is determined by the *mec*A gene, which is a part of an additional DNA region, the staphylococcal cassette chromosome *mec* (SCC*mec*).[87] Mechanisms of MRSA dissemination are the spread of individual MRSA clones and horizontal transfer of SCC*mec* among *S. aureus* and other staphylococci.[87] Methicillin-resistant strains of *S. aureus* were first reported in 1961, and subsequently outbreaks of MRSA infections occurred worldwide forming a significant proportion of all *S. aureus* isolates in many countries.[40,49,86] Widespread antibiotic use, presence of intravascular catheter, severe underlying disease, prolonged hospitalization, and poor adherence to infection control precautions have contributed to the rise in MRSA rates.[76,78,88]

The incidence and prevalence of MRSA varies widely between countries, regions and even hospitals.[54,89,90] In a survey from the SENTRY Antimicrobial Surveillance Program, geographic variations of MRSA prevalence from all sites of infections were found, as follows: Western Pacific region 46%, Latin America 35%, United States 34%, Europe 26%, and Canada 6%.[40] In Europe, the proportion of MRSA rates ranged from ≤2% to 54%, and among Western Pacific countries from 24% to 70%.[40,89] Finland is a country with a very low prevalence of methicillin resistance. For

many years prevalence of MRSA was less than 1% among invasive infections. However, during 2004 increased incidence in the number of MRSA bacteremias was observed but its prevalence still remained below 3%.[2,90,91]

MRSA has been originally confined to nosocomial infection, with only rare community-associated cases. The prevalence of hospital-acquired MRSA isolates increased progressively in the United States from 2% in 1975 to 35% in 1991.[92] The rate among bloodstream MRSA isolates during 1997-1999 was even higher, up to 45%.[40] MRSA has accounted for over 60% of all isolates in intensive care units (ICU) in the United States, especially among older patients.[93] However, a major change in the epidemiology of staphylococcal infections is the rapid emergence of community-acquired MRSA strains which frequently produce dangerous exotoxins (e.g., Panton-Valentine leukocidin).[94,95] According to a recent meta-analysis,[96] the pooled prevalence rates of community-associated MRSA isolated from hospitalized patients were 30% to 37%. These infections usually involve the skin, and outbreaks have been described among prisoners or IDUs, and among patients without established risk factors such as young children, military recruits, or competitive sports participants.[97-99]

Generally, nosocomial MRSA isolates are multiresistant and clonal, whereas community-associated MRSA strains are pauciresistant and more polyclonal.[86] Glycopeptide agents have been considered effective antibiotics against multidrug-resistant *S. aureus*. Therefore, reports of staphylococci with reduced susceptibility to these agents are alarming. The first clinical isolate of vancomycin-intermediate *S. aureus* was described in Japan in 1997,[100] and since then these strains have continued to cause healthcare-associated infections worldwide. More recently, there have been single reports of vancomycin-resistant *S. aureus* infections which have not been observed in Finland.[104,105]

2.2. Risk factors for *Staphylococcus aureus* bacteremia

Staphylococcus aureus colonization

Many healthy adults are persistently or intermittently colonized with *S. aureus* in their anterior nares. Approximately 20% of individuals are persistent nasal carriers, 30% are intermittent carriers, and 50% are non-carriers.[106,107] Nasal carriage of *S. aureus* is one of the most important risk factors for nosocomial and surgical site infections.[86,107] Some subgroups such as IDUs, patients undergoing hemodialysis or peritoneal dialysis, and patients with diabetes, human

immunodeficiency virus (HIV) or recurrent skin infections are at increased risk for skin and nasal colonization with *S. aureus*.[108-112] Studies among IDUs have shown that injection of contaminated drug and inhalational drug use may support nasal *S. aureus* colonization, probably by damaging the nasal mucosa.[63,113]

In recently published studies, nearly 80% of nosocomial SAB were due to the same phage type of *S. aureus* strain isolated from the patients` anterior nares.[107,114] Decolonization with mupirocin has been shown to prevent staphylococcal disease in dialysis and surgical patients.[115,116] However, recent clinical trials in non-surgical and orthopedic patients uniformly failed to confirm these results.[117,118]

Comorbid conditions and predisposing factors

Several factors have been suggested to increase the risk for invasive *S. aureus* infections. A high proportion of patients with SAB have underlying diseases such as cardiovascular disease, chronic obstructive pulmonary disease, diabetes mellitus, malignancy, chronic renal failure, HIV infection, or hepatic cirrhosis.[1,51,84,119-121] Only 3% to 5% of patients have had no underlying disease.[1,84] Furthermore, recent surgery, prosthetic devices, or the presence of intravascular catheters are important risk factors.[76] Older age (>60 years), male sex, alcohol abuse, hyponatremia, anemia, immunosuppressive treatment, injection drug use, preceding trauma, previous hospitalization, or prolonged treatment in ICU predispose to SAB.[28,44,51,79,119,120,122] Patients with chemotactic defects (e.g., Job`s syndrome) and defects in phagocytosis are also at increased risk for staphylococcal disease.[86]

Risk factors for endocarditis and complicated bacteremia

Historically, most cases of IE have occurred in association with community-acquired SAB.[84,123] Underlying cardiac diseases have remained one of the most important risk factors for endocarditis among patients with SAB.[124] Classic risk factors such as rheumatic heart disease are now being replaced by new ones, including IDUs, elderly patients with degenerative valve sclerosis, hemodialysis patients, and patients with an intravascular catheter or prosthetic valve.[21,65,86,125,126] In addition, previous IE, mitral valve prolapse, unknown portal of entry, immunosuppression, and hospital-acquired bacteremia have been related to predisposing conditions for endocarditis.[26,57,58,65,127,128]

A variety of strategies have been used in attempt to identify patients who develop metastatic infections secondary to SAB. In recent studies, the most important risk factors for complicated SAB

were community-acquisition, hemodialysis,[79,130] one or more underlying diseases,[128] persistent bacteremia or fever for more than 72 hours,[16,65,131,132] C-reactive protein (CRP) level >100 mg/L,[128] the presence of a permanent foreign body,[79] or a failure to remove an infected catheter.[79,133] The impact of MRSA in complicated SAB has been evaluated in some studies. Patients with MRSA bacteremia did not have a higher rate of metastatic infections,[6,16,134,135] although in one study methicillin resistance was independently associated with an increased risk for metastatic infections in intravascular catheter-associated SAB.[79] The genetic properties of MRSA isolates may produce more virulent phenotypes in cases of catheter-associated bacteremia. Treatment of MRSA-infected patients with vancomycin has also been related to clinical failure and prolonged bacteremia.[136,137]

2.3. Clinical manifestations in *Staphylococcus aureus* bacteremia

2.3.1. Classification of infection foci

The initial infection focus for SAB is most often skin or soft tissue infection such as a wound or furuncle. From this local infection, the bacteria will spread superficially, and a bloodstream infection may follow.[119] Virtually every organ may be infected by *S. aureus*.[32] However, varying definitions of the infection foci are used in the literature. Nolan and Beaty first proposed that infection foci in SAB can be divided into two groups.[123] Criteria were established for the designation of lesions in various body sites as primary (i.e., portal of entry) or secondary foci (i.e., metastatic infection) (**Fig. 1**).

Primary focus   **Bacteremia**   **Secondary focus**

Skin
Postoperative wound
Intravenous device
Lung
Urinary tract
Unknown

Endocardium
Bone
Joint
Meninges
Other
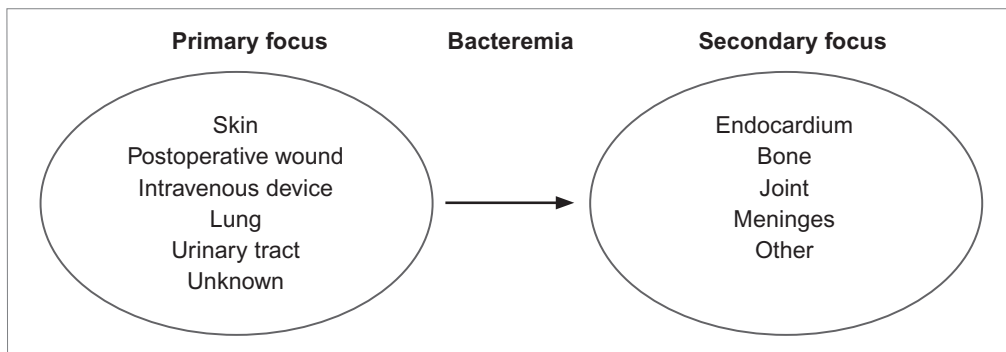
Figure 1. Infection foci of *Staphylococcus aureus*. A primary *S. aureus* infection may lead to bacteremia resulting in a secondary infection.[120]

A localized skin lesion or other superficial staphylococcal infection are considered as a source (primary focus) for SAB if signs and symptoms, and physical findings of this infection precede

the bacteremia.[123,128,138] The presence of an intravenous catheter has been recognized as a portal of entry.[8,84] Respiratory and urinary tracts are considered as primary foci for SAB when the signs and symptoms of infection typically associate with the bacterial culture results.[8,123,139,140] Furthermore, when the portal of entry is unknown, SAB is defined as primary.[84] However, the absence of a clinically identifiable source for SAB has been thought to be an important predictor for metastatic infections.[84,123]

Endocarditis, osteomyelitis, septic arthritis, and meningitis are always categorized as secondary foci caused by hematogenous spread of SAB, unless there is evidence of direct inoculation of the bacteria like surgery or trauma.[7,17,121,123] Isolation of *S. aureus* from urine is considered secondary to SAB,[141] if the phage type of the organisms isolated from urine and blood matches.[84] Pneumonia can also be hematogenous when caused by embolization of infected thrombotic material from tricuspid vegetations.[8] In addition, deep-seated abscess and infection of permanent foreign bodies are generally classified as metastatic infections (i.e., secondary foci).[11,16,79,128,142]

In some studies, SAB has been classified as uncomplicated or complicated. Uncomplicated SAB is defined as catheter-associated bacteremia or other than catheter-related bacteremia when there is no evidence of secondary foci or recurrent SAB within three months.[16,79] Complicated SAB has been considered as bacteremia with secondary foci or recurrent SAB within three months, and/or with other clinical findings such as shock, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), acidosis, involvement of central nervous system (CNS), or evidence of an embolic or autoimmune event.[1,9,16,39,129,143]

It may be difficult to distinguish between primary and secondary foci, or whether SAB is uncomplicated or complicated. In one review, infections caused by *S. aureus* were defined as cutaneous and deep infections.[32] These deep infections consisted of e.g., bacteremia, osteomyelitis, septic arthritis, deep-seated abscess, endocarditis, pneumonia, and foreign body or CNS infection to replace classification for secondary foci. The definition for deep infections described above was used in our trials.

2.3.2. Frequencies of infection foci
In many articles, primary foci have been combined together as a group and have not been separated. The frequency of primary foci varies from 35% to 85% of cases according to different studies.[1,8,84,143,144] In addition, the source of SAB is unknown in 2% to 58% of patients, suggesting

that *S. aureus* may invade the bloodstream without clinically superficial infection.[6,7,16,65,143] The reported frequency of metastatic infections in SAB varies widely from 10% to 53%.[16,84,121,145] These different observations might be partly explained by underdiagnosis or different definitions of the metastatic infections such as secondary foci, complicated bacteremia or deep infections.[124] Metastatic infections have usually been diagnosed within the first two weeks, and even 74% of them are already present at the time of hospitalization.[16,39]

2.3.3. Skin and soft tissue infections

Intact skin is normally an excellent barrier against *S. aureus*, but when this barrier is broken or foreign body implanted, infection can easily be established.[119] When bacteria have penetrated into the skin, they can disseminate to more profound, normally sterile sites. The typical pathological finding of staphylococcal disease is a pyogenic exudate or an abscess. *S. aureus* infections of the skin and soft tissues are classified according to the anatomic structure: (1) infection of the epidermis is represented by impetigo; (2) infection of the superficial dermis by folliculitis; (3) infection of the deep dermis by furuncles, carbuncles, and hidradenitis suppurativa; and (4) infection of subcutaneous tissues by erysipelas, cellulitis or fasciitis with increasing depth of infection.[86,146,147] Furthermore, *S. aureus* is a major cause of surgical wound infections in hospitalized patients,[148] and soft tissue abscesses are the most common complications among IDUs.[113]

2.3.4. Catheter-related bacteremia

The presence of a central venous catheter is an important risk factor, with up to 56% of all episodes of bacteremias originating from intravascular catheters, especially in hospitalized patients.[11,149] The presence of a permanent foreign body (i.e., either intravascular or non-catheter device), hemodialysis dependence, and methicillin resistance of the *S. aureus* strain have shown an increased risk for metastatic infections and treatment failures in catheter-associated SAB.[11,79,142] Therefore, current guidelines suggest that non-tunneled central venous catheters should be removed immediately when they are found to be the source of SAB.[11,79,133,150] A tunneled (i.e., Hickman catheter) or implantable device should be removed if there is purulence or erythema at the exit site or along the tunnel, evidence of a pocket infection, or if it is associated with a complicated deep-seated infection.[146] In a meta-analysis of intravascular catheter-related SAB, 24% of patients developed metastatic infections or relapses.[151] Persistent bacteremia or fever for more than 72 hours after removal of the catheter have been related to secondary foci.[16,152]

Although the potential association between catheter-related SAB and endocarditis has been recognized for decades, the clinical significance of this phenomenon has only recently become more evident. The incidence of *S. aureus* endocarditis in bacteremic patients with central intravenous catheter varies widely from 23% to 61%.[12,124,153] Fowler et al recently showed that 23% of patients with catheter-associated SAB had an endocarditis documented by transesophageal echocardiography (TEE) in the absence of clinical or transthoracic echocardiographic findings.[153]

2.3.5. Endocarditis

Diagnostic criteria

Various diagnostic criteria have been established for endocarditis. In the literature before 1960, endocarditis was associated with SAB in 64% of cases.[121,154] In these studies, the diagnosis of IE was confirmed by autopsy in many instances. In 1976, Nolan and Beaty reported criteria for predicting the presence of endocarditis in patients with SAB. According to them, endocarditis was more likely in (1) community-acquired bacteremia, (2) when the focus was unknown and (3) when metastatic infections were present.[123] In 1981, modified criteria by von Reyn and coworkers improved the diagnostic specificity in endocarditis,[155] but these criteria did not use echocardiographic findings in the case definitions. In 1994, new diagnostic Duke criteria based on a combination of histopathology, microbiological and echocardiographic imaging were introduced.[156] These criteria stratified patients with endocarditis as definite, possible or rejected. However, a modified version of the Duke criteria with definite or possible IE was proposed in 2000 to detect more accurately an endocarditis in *S. aureus*-associated bacteremia.[22,62,75]

Echocardiography has become widely used to determine the diagnosis of endocarditis. Transthoracic echocardiography (TTE) is recommended for high-risk patients with endocarditis such as community-acquired SAB, injection drug abuse, valvular heart disease, prior IE, an unknown source of infection, and persistent bacteremia.[60,65,69] The overall sensitivity of TTE in detecting vegetations may be less than 60% to 70%.[157-160] Because of the limited sensitivity of TTE, a negative result cannot exclude the diagnosis of IE.

TEE is more expensive and invasive, but it has increased sensitivity for detecting vegetations of 75% to 95% but still maintains specificity of 85% to 98%.[126,160] In most studies of SAB, TTE has been performed in a high proportion of patients ranging from 49% to 76%, whereas only 12% to 42% of patients underwent TEE.[7,16,60,128] Fowler et al showed that vegetations were seen only

by TEE in 56% of patients with endocarditis due to SAB,[12] and therefore the initial use of TEE is recommended in patients in the presence of prosthetic cardiac valves or other permanent cardiac devices, catheter-associated bacteremia, and a suspicion of cardiac complications such as abscesses.[76,126,153,157,161]

Clinical picture of Staphylococcus aureus endocarditis

The clinical picture of endocarditis is complex and can be divided into following processes: (1) valve infection and local cardiac complications; (2) extracardiac deep infections such as metastatic foci; (3) septic embolism to any organ; and (4) circulating immune complexes.[162] Endocarditis in SAB is characterized by a rapid onset with high fever and the absence of physical findings of IE on the initial presentation in contrast to subacute endocarditis caused by streptococci.[28,76,157] Patients with *S. aureus* endocarditis may respond slowly and become afebrile not until five to seven days after the institution of therapy. In acute native valve endocarditis, heart murmur is noted in only 45% of cases on initial evaluation.[157] The mean duration of symptoms before therapy in *S. aureus* endocarditis is only three days.[11,163] Myocardial abscesses, purulent pericarditis, and valve ring abscesses are common local cardiac complications.[164] Extracardiac deep infections have been associated with SAB in left-sided endocarditis with an incidence ranging from 40% to 76%.[12,67,69,164]

Congestive heart failure and systemic embolic events have the greatest influence on the prognosis of endocarditis. Systemic thromboembolic events (e.g., spleen, kidney, liver, or cerebral) occur in 21% to 50 % of patients, especially among those with left-sided involvement and prosthetic heart valve.[68,126,165-170] In addition, mitral valve involvement and large vegetations (>10 mm) have been associated with an increased risk for embolization.[166,171] Most systemic thromboembolic manifestations are observed on presentation or within the first two weeks, and the occurrence of vascular phenomena decreases after initiation of effective antibiotic therapy.[27,165,172,173] Cerebral emboli or ischaemic stroke may result in hemiparesis, whereas mycotic aneurysms are usually silent, but can lead to intracerebral or subarachnoid hemorrhage.[162]

The peripheral septic embolic manifestations in endocarditis are most frequently petechiae and occasionally Janeway lesions, which are painless, and hemorrhagic spots found on the palms and soles.[126,157] Renal insufficiency can be a result of immune complex-mediated glomerulonephritis and occurs in less than 15% of patients with IE.[157] Other immunological phenomena such as Osler`s nodes (tender, subcutaneous nodules in fingers and toes) and Roth`s spots (retinal hemorrhagic lesions) are also observed in endocarditis.[126,164]

The clinical picture of endocarditis in nonaddicts differs from that in IDUs regarding different location of infection, clinical manifestations and prognosis.[120] Nonaddicts have more often an underlying heart disease, most patients are older than 50 years, and *S. aureus* primarily involves the left side of the heart in 80% of nonaddicts.[60,162] Furthermore, the mitral valve is affected more frequently than aortic valve, and only a few patients have right-sided endocarditis, or both mitral and aortic valve involvement simultaneously.[12,60]

In a recent multicenter survey published in 2005, 81% of cases with *S. aureus* endocarditis had native valve involvement, whereas prosthetic valve was involved in 17% of cases.[18] However, the risk of endocarditis in patients with a prosthetic valve who develop SAB is high, up to 51%.[174,175] The overall risk is similar for both mechanical and bioprosthetic valves as well as for aortic and mitral valve prostheses.[174,176] Prosthetic valve infection has been called early when symptoms begin within 60 days of valve surgery and late with onset thereafter.[157] The risk of development of prosthetic valve infection has been thought to be highest within the first three months after operation,[177] but in one study the risk was independent of the age of the cardiac valve device.[174] The clinical features of prosthetic valve infection are similar to those in native valve endocarditis with left-sided involvement although the risk for thromboembolic events is higher in prosthetic valve endocarditis.[172,177]

2.3.6. Bacteremia and endocarditis in injection drug users

S. aureus is the most common cause of bacterial infections among IDUs.[18,178] *S. aureus* infections in addicts range from cutaneous soft-tissue abscesses to life-threatening bacteremia or endocarditis.[178,179] IDUs are more likely to develop bone and joint infections, particularly vertebral osteitis, than nonaddicts.[63,179] The factors that seem to contribute to the high prevalence of staphylococcal disease among IDUs include the pathogen, the host, the drug, the drug-use environment, and drug using habits.[113,179] The source of *S. aureus* may be endogenous (the drug user´s own flora) or external (contaminated drugs, drug adulterants, or paraphernalia).[180-183]

Colonization with *S. aureus*, injection of heroine or cocaine, HIV infection, history of previous IE, or skin abscesses have been recognized as risk factors for endocarditis among IDUs.[178,184-187] Most addicts with IE are younger than 40 years of age with male sex predominance.[18,21,64,71] Almost two thirds of IDUs have no history of severe underlying conditions or predisposing heart diseases.[19,27,71,188,189]

A variety of theories have been proposed to explain the increased prevalence of right-sided endocarditis among IDUs but still there is limited understanding of its pathogenesis. Potential explanations include damage to right-sided endothelium by repeated exposure to injected

particulate matter, vasospasm and inflammation caused by injected diluents or drugs. Furthermore, drug-induced pulmonary hypertension with increased right-sided intracardiac turbulence or factors relating to the microorganism itself have also been thought to explain the common right-sided involvement in IDUs.[179,190,191]

The tricuspid valve is affected in 70% to 90% of cases among IDUs followed by the mitral and aortic valves.[19,20,22,71,186,192,193] In the literature, the prevalence of left-sided involvement in addicts has ranged from 5% to 19%,[19,193-195] but in some studies it has been even higher, up to 57%.[20,188,196] Both sides of the heart are involved simultaneously in only a few cases.[19,188] *S. aureus* endocarditis in IDUs is reported to be associated less frequently with extracardiac deep infections and systemic arterial emboli or strokes, probably due to right-sided involvement.[19,21,71,187,194,197] However, in a previous report there were no differences in maximum temperature, or leukocyte count between addicts with and without endocarditis.[179] Septic pulmonary embolism occurs in up to 87% of cases of right-sided endocarditis[18,71,185,188,195] but peripheral septic skin manifestations are usually absent in accordance with right-sided involvement.[187]

2.3.7. Other deep infections

Osteomyelitis and septic arthritis

Bone and joint infections are the next most common infection sites caused by *S. aureus* after skin and soft tissue infections.[198] Long bones may be involved following hematogenous dissemination of *S. aureus*, but osteitis in these locations is typically a result of contiguous spread from a traumatic wound or infected ulcer and is most often seen in diabetics with vascular disease.[86] Acute hematogenous *S. aureus* osteomyelitis in lumbar or thoracic vertebrae is observed more often, in up to 19% of patients, especially among the elderly. Furthermore, a paraspinous or epidural abscess is frequently associated with the vertebral osteomyelitis.

Septic arthritis is generally a result of surgical intervention and hematogenous spread or may be iatrogenic in the case of joint puncture or arthroscopy.[17,201] The most common joints during the course of SAB are the knee, hip, elbow, shoulder, and interphalangeal joints.[17] The risk of joint infection among patients with SAB increases in those who are immunosuppressed or have rheumatoid arthritis.[86] *S. aureus* is also the most frequent cause of septic arthritis in children.[198]

Meningitis

S. aureus meningitis is often a result of trauma, neurosurgical procedure or infection of a ventricular shunt. Meningitis due to hematogenous spread is reported in only 1% to 9% of bacteremic cases.[1,12,16,134,202,203] Meningitis caused by staphylococcal bacteremia is usually a part of disseminated infection with other secondary foci such as endocarditis or osteomyelitis.[86,162]

Patients with hematogenous meningitis are older, have chronic underlying diseases, and their prognosis is poor with a high mortality rate of 56%.[203]

Pneumonia

Pneumonia due to *S. aureus* constitutes 1% to 10% of cases of community-acquired pneumonia[204-206] and up to 30% of cases of nosocomial pneumonia.[207] Pneumonia may be caused by aspiration or hematogenous spread due to a release of infected thrombotic material from the venous system or from infected tricuspid vegetations frequently seen in IDUs.[120,208] In recent studies, pneumonia during the course of SAB is observed in 6% to 34% of patients.[7,8,121,144] A necrotizing pneumonia related to the toxin-producing Panton-Valentine leukocidin (PVL) strain is a new entity. It occurs in healthy children and young adults, may rapidly progress to acute ARDS, and carries a high mortality rate.[209]

Urinary tract infection

S. aureus can cause ascending urinary tract colonization, and primary infection is observed mainly among long-term care patients.[140] Persistent urinary staphylococcal colonization is associated with high risk of bacteremia. Therefore, it is important to recognize predisposing factors for primary staphylococcal bacteriuria which include nosocomial causes (e.g., indwelling catheters or surgery) and obstructive disease (e.g., prostatic hyperplasia or stricture).[210] Paradoxically, *S. aureus* bacteriuria can be a consequence of bacteremia with secondary hematogenous spread to the kidneys reported in up to 7% of SAB.[8,140,211,212] Risk factors for *S. aureus* bacteriuria secondary to SAB are chronic underlying diseases like diabetes and cancer, or hemodialysis, injection drug abuse and the presence of foreign bodies.[8,210]

Virtually any organ may be infected by *S. aureus* due to hematogenous dissemination. Other common deep infections such as deep-seated abscesses, pleural empyema, mediastinitis, pericarditis, septic bursitis, pyomyositis, or septic thrombophlebitis can occur as clinical manifestations during the course of SAB.[86]

Foreign body infections

An increasing number and variety of prosthetic devices are presently implanted in patients, and *S. aureus* is the second leading cause of prosthetic joint infections after coagulase-negative staphylococci.[213,214] In a recent study, 42% of orthopedic devices and 45% of cardiac devices (e.g., cardiac pacemaker or implantable cardioverter-defibrillator) were infected in patients with SAB.[215] In addition, the incidence of other deep infections (i.e., endocarditis, osteomyelitis, and deep-seated abscesses) was as high as 49% in patients with an orthopedic device.[215]

Hematogenous seeding from a remote infection focus is a relatively rare mechanism of vascular graft infection with the highest risk during the first four to six postoperative months.[214]

2.4. Mortality in *Staphylococcus aureus* bacteremia

2.4.1. Predictors for mortality and poor prognosis

The mortality of SAB was extremely high up to 82% in the preantibiotic era (**Fig. 2**).[216] Later, between 1950 and 1990, the mortality rate decreased and varied in different surveys from 24% to 58%.[5,122,217-220] Today, the mortality of SAB is lower, but still high ranging between 7% to 39%.[3,6,9-14] A variety of definitions based on either clinical judgments and/or time from SAB to death has resulted in a wide range in mortality. The low mortality rates have been based on the definition of death within seven days after the onset of SAB including clinical or microbiological evidence of *S. aureus* infection at the time of death (i.e., mortality due to SAB).[12,45,221,222] In other studies, higher mortality rates have been reported within two to five weeks after the onset of bacteremia (i.e., in-hospital or overall mortality).[3,7,8,120,135]
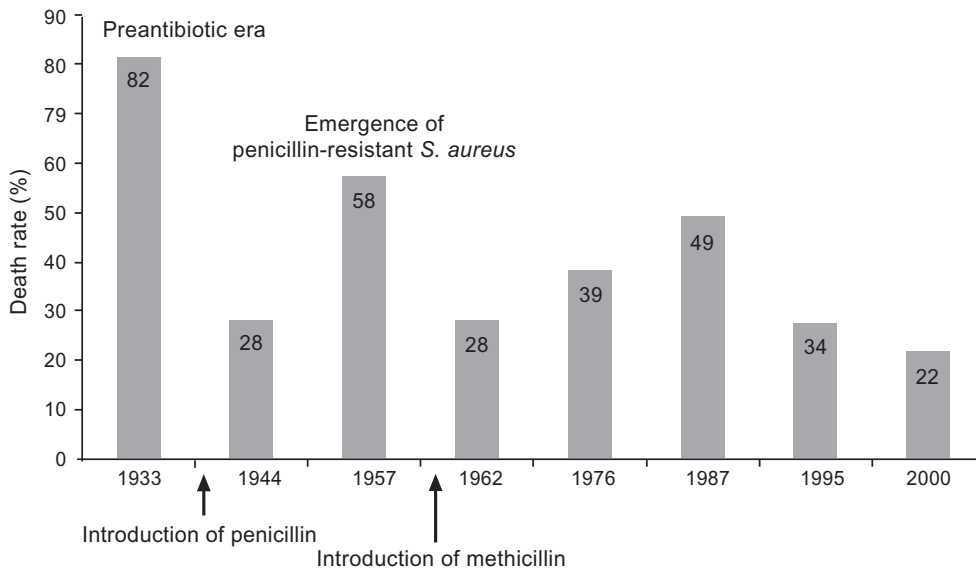
Figure 2.   Mortality rate of *Staphylococcus aureus* bacteremia at various time points.[8,10,121]

Some data suggest that hospital-acquired bacteremia is associated with higher death rates.[3,5,80] This might be due to older age and more severe comorbid conditions in hospitalized patients. However, the overall mortality in many recent studies has been shown to be approximately similar both for community-acquired and nosocomial cases.[7,8,12,83,85] Serious underlying diseases,[14,84,121]

age over 60 years,[7,10,123] severe sepsis,[1,68] persistent bacteremia,[65] inapproriate empirical antibiotic treatment and failure in eradication of an infection focus have been associated with higher mortality in SAB.[3,6,8,14,17] Even less potent or too short treatment might carry a risk for a poor prognosis. According to a recent study,[8] a total daily dose <4 g of dicloxacillin sodium and the duration of antibiotic treatment for less than 14 days were significantly related to fatal outcome. Higher mortality has been observed also in patients with thromboembolic events and deep infections,[16,18] such as pneumonia, meningitis and endocarditis.[5,7,10,13,134] Furthermore, an unknown source of SAB has been identified an independent risk factor for death.[3,7,145] The high level of Acute Physiology and Chronic Health Evaluation (APACHE II) score in SAB was recently found to predict for poor prognosis.[7,144,223] Other factors associated with fatal outcome include hyperbilirubinemia (>40 μmol/L), elevated level of serum creatinine (≥200 μmol/L), thrombocytopenia (<100 x 10^9/L), acidosis (blood pH<7.3), leukocytosis or leukopenia (>10.0 x 10^9/L or <3.0 x 10^9/L), and granulocytosis or granulocytopenia (>8.0 x 10^9/L or <1.0 x 10^9/L).[1,5]

2.4.2. Catheter-related bacteremia

An increasing percentage of SAB is related to catheterization.[11,149] The mortality rate in intravenous catheter-associated SAB has generally been low ranging from 5% to 10% but in some studies up to 33%.[39,144] According to a current meta-analysis,[151] the pooled mortality rate for catheter-related SAB was 15% regardless of duration of therapy.

2.4.3. Endocarditis among nonaddicts

The overall mortality in patients with *S. aureus* endocarditis is high varying between 30% to 46%,[18,60,65,66,143,224] but an even higher rate of 71% was published in 1986.[124] Higher mortality has been associated with left-sided involvement, older age, rapidly fatal underlying diseases, pre-existing heart valve diseases, renal failure, severe sepsis, prior hospitalization within 30 days of onset of SAB, persistent bacteremia, and the presence of a prosthetic valve.[7,21,65,68,189,224,225] In a recent study on 566 patients with native valve *S. aureus* endocarditis,[18] additional important predictors for mortality were heart failure, formation of periannular abscess, aortic or mitral valve vegetations, CNS thromboembolic manifestations and absence of surgical therapy.

The prognosis of prosthetic valve endocarditis has improved since the 1990s, although the mortality is still about 50%.[76,174] Improved survival has been related to many factors, including early surgical intervention, recognition of the need for multidrug therapy, use of TEE for early

identification of endocarditis and cardiac complications, and improvements in the management of critically ill patients hospitalized in ICUs.[174,177]

2.4.4. Endocarditis associated with injection drug abuse

In the past two decades, endocarditis has become one of the most prevalent causes of death in IDUs. However, the mortality of *S. aureus* endocarditis among this patient group is remarkably low varying between 2% to 12%.[19-21,189,193,224] The favourable outcome in addicts is still not completely understood but is generally explained by host factors such as younger age, lack of pre-existing heart disease or other underlying diseases, and right-sided involvement.[18,19,21,157,190,224] The latter factor is supported by findings of better sterilization of right-sided valve vegetations in experimental endocarditis as compared to left-sided valves.[226,227] Furthermore, the density of bacteria in infected tricuspid vegetations was smaller as well. Only addicts with ARDS, tricuspid valve vegetations over 2 cm in size and left-sided involvement have had higher mortality ranging from 20% to 33%.[76,185,228] In addition, IDUs with severe immunosuppression such as acquired immunodeficiency syndrome (AIDS) present a fatal outcome more frequently than in immunocompetent patients.[195]

2.4.5. Bacteremia due to methicillin-resistant *Staphylococcus aureus*

The contribution of methicillin resistance to the morbidity and mortality in SAB is controversial. In a recent meta-analysis[15] and in some well-designed studies,[3,65,134,229] MRSA bacteremia was significantly associated with higher mortality than bacteremia caused by methicillin-sensitive *S. aureus* (MSSA) strains. Furthermore, patients with endocarditis due to MRSA are significantly more likely to have persistent bacteremia than those with endocarditis caused by MSSA.[21,65] However, several other studies suggest that MRSA bacteremia is not associated with increased risk for death.[6,7,14,43,135,230] Because patients infected with MRSA tend to be older,[3,45] have more often severe underlying diseases,[6] septic shock or pneumonia[3] than patients infected with MSSA, evaluations on the impact of MRSA on patient outcome should be adjusted for these confounding factors.[16,135,231] Therefore, the association between MRSA and mortality may be partially explained by inherent differences between the patient groups rather than methicillin resistance itself. Specifically, the difference in mortality between MRSA and MSSA may only be evident in certain subset of patients, such as severely ill ICU patients.[229,230] In addition, different antibiotics are used to treat MRSA and MSSA infections,[230] and patients with MRSA are at increased risk for delayed therapy.[232] Some clinical evidence suggests that vancomycin would be inferior to beta-lactam antibiotics in the treatment of serious staphylococcal infections such as endocarditis.[136,137] Furthermore, a growing amount of evidence suggests that accessory gene

regulator (*agr*) group II polymorphism in MRSA might be predictive of failure of vancomycin therapy.[233]

2.5. Recurrence of *Staphylococcus aureus* bacteremia

Recurrent SAB is a common phenomenon. A second episode of SAB may represent as a relapse confirmed by the same resistance pattern and pulsed-field gel electrophoresis (PFGE) type of the two consecutive infecting *S. aureus* strains, or as a reinfection unrelated to prior staphylococcal infection.[234] Recurrent episodes of SAB have ranged from 9% to 23% in studies with a median follow-up time of three to six months.[6,8,11,13,16,65,235] According to a recently published study,[235] relapse of SAB after completion of antibiotic therapy occurred earlier than reinfection (median, 36 versus 99 days).

Recurrences of SAB are primarily relapses and are associated with an unremoved foreign body, hemodialysis, native heart valve endocarditis, liver cirrhosis, and vancomycin therapy.[150,189,234-236] Vancomycin treatment may also predispose to prolonged bacteremia.[237] Furthermore, a total daily dose of dicloxacillin less than 3 g, short duration of therapy in SAB with deep infections, persistent bacteremia over three days, and underecognition of osteomyelitis or endocarditis are all risk factors for a recurrent SAB.[8,13,16,129,235,238] Recurrent endocarditis is common especially in addicts, and the median interval between episodes is far shorter among IDUs compared with nonaddicts.[170] Thus, all patients with relapsing SAB are recommended to undergo TEE.[235] Recent studies have also demonstrated that consultation of an infectious disease specialist can improve remarkably the clinical outcome and reduce the number of relapses for patients with SAB.[7,11,84,143]

2.6. Treatment of *Staphylococcus aureus* bacteremia

2.6.1. Standard antibiotic therapy

In Finland, the frequency of bacteremic MRSA infections is less than 3%,[90,91] and therefore the parenteral semisynthetic penicillins (cloxacillin or dicloxacillin) constitute the basis of standard therapy in SAB.[28,239] The usual dose in adults is 2 g of oxacillin or dicloxacillin every four to six hours.[24,26] Such doses of dicloxacillin can cause significant thrombophlebitis and administration through a central venous line is usually necessary. When patients have penicillin allergy without anaphylaxis, first- or second-generation cephalosporins, or clindamycin can be used.[26,62,157] Clindamycin is bacteriostatic and related to an increased risk of relapses, and therefore it is

not recommended for treatment of endocarditis.[120,240] Instead, it can be used in osteomyelitis due to its excellent bone penetration.[50] Vancomycin has been associated with a high frequency of clinical failures in several studies,[11,235,241-243] although this finding is controversial.[16] Thus, vancomycin should be used only in MRSA strains or in patients intolerant or allergic to penicillins and cephalosporins.[13,244] Experience with newer antistaphylococcal agents (e.g., linezolid, quinupristin-dalfopristin, daptomycin, or tigecycline) is still limited, especially in endocarditis, and their ability to prevent persistance and relapse of SAB is unknown.[27,237,245,246] These antibiotics are indicated for the treatment of MRSA infections and patients who are infected with a strain having reduced susceptibility to vancomycin, or who fail on or are intolerant of conventional therapy.[24,26]

2.6.2. Antibiotic therapy in catheter-related and uncomplicated bacteremia

The most common type of uncomplicated SAB is catheter-related bacteremia. The standard antibiotic treatment consists of a beta-lactam based regimen as monotherapy in patients with bacteremia due to MSSA.[242] The optimal duration of antibiotic therapy for central intravenous catheter-associated bacteremia has been controversial and questioned for decades. However, recent studies have suggested that the risk for endocarditis and other deep infections in patients with localized catheter-related SAB is low enough to recommend short-course parenteral therapy (10 to 14 days) when the infected catheter has been removed.[121,133,149,161,242,247] Patients with predisposing valvular abnormalities, superficial non-removable infection focus, and persistent bacteremia or fever for more than 72 hours after removal of the catheter have more often deep infections.[16,152,248] In these cases, longer parenteral therapy of four to six weeks is recommended. Furthermore, TEE has been shown cost-effective in the evaluation of possible endocarditis and in determination of the duration of therapy in catheter-related bacteremia.[161,249] For patients with uncomplicated SAB other than catheter-related bacteremia, two weeks parenteral therapy followed by two weeks oral treatment has been suggested.[250,251]

2.6.3. Studies on combination antibiotic therapy

Aminoglycoside combined with standard therapy

Data on the effect and recommendations for various antibiotic combinations are variable in SAB with metastatic infections. Combination therapy has been used to increase bactericidal activity or to prevent development of antimicrobial resistance. Specifically, the combination of aminoglycosides and beta-lactams produces a synergistic effect and increases bacterial killing *in vitro* and in animal models of *S. aureus* endocarditis.[28,164,252] However, the benefits of combination therapy with an aminoglycoside have not been convincingly established in human

clinical trials. [253-256] In one prospective study consisting of patients with mainly left-sided *S. aureus* endocarditis,[257] a more rapid clearance of bacteremia was achieved with the combination of gentamycin and nafcillin than with nafcillin therapy alone. However, mortality or cardiac complications were not reduced, but in contrast gentamycin-associated nephrotoxicity was evoked.

Rifampicin combined with standard therapy

Recommendations for combination therapy including rifampicin are controversial although it is widely used in SAB with deep infections and when the response to standard antistaphylococcal antibiotic therapy alone is either poor or slow.[28,32] Rifampicin shows excellent antistaphylococcal activity against MSSA and MRSA strains including penetration into cells and biofilms, and ability to kill phagocytosed bacteria.[33,34,239,258] In a recent study, rifampicin effectively eradicated *S. aureus* also in non-phagocytic cells *in vitro*,[259] which is suggested to be valuable in the treatment of invasive *S. aureus* infections.

Specifically, the combination of oxacillin and rifampicin had a synergistic action *in vitro* when the concentration ratio of oxacillin to rifampicin was low, whereas antagonism occurred with higher ratios.[29] Rifampicin and fluoroquinolone in combination had also an antagonistic effect in *S. aureus* endocarditis in animals when administrated on short-term treatment (four to six days).[33,260,261] However, two other experimental animal studies showed improved results with combination therapy with rifampicin and nafcillin or vancomycin as compared to single-drug treatment in chronic staphylococcal osteomyelitis although the drug combination was antagonistic *in vitro*.[262,263] Antagonism between rifampicin and other antimicrobials *in vitro* has substantially hampered the clinical acceptance of rifampicin in the treatment of staphylococcal infections. Furthermore, resistance in staphylococci invariably develops during therapy if rifampicin is used alone.[23,29,264-266] Ideally, the companion drug should exhibit pharmacokinetics similar to rifampicin without an antagonistic interaction.[267]

Only limited human data are available to support the use of rifampicin in severe *S. aureus* infections. Some small randomized clinical trials suggested that adding rifampicin to semisynthetic penicillin in patients with severe *S. aureus* infections improved clinical cure and bacteriological eradication whereas no effect in mortality was seen.[30,31,268,269] However, the combination of rifampicin and vancomycin in a study of MRSA endocarditis had no significant advantage in clinical response over vancomycin alone.[137]

Fluoroquinolones in the treatment of staphylococcal infections

The fluoroquinolones are a relatively new class of antimicrobials with good tissue and intracellular penetration, and with an ability to kill intracellular bacteria.[37,267,270,271] Fluoroquinolones exhibit high serum and tissue concentrations after oral administration, and have a low side-effect profile.[272,273] Differences in the activity of the fluoroquinolones *in vitro* primarily form the basis of their classification. The older ones (e.g., ciprofloxacin, norfloxacin, and ofloxacin) predominantly have activity against gram-negative bacteria.[37] The newer fluoroquinolones (e.g., levofloxacin, trovafloxacin, moxifloxacin, gemifloxacin, and gatifloxacin) exhibit improved activity against gram-positive organisms and also have improved pharmacokinetic properties allowing dosing more seldom.[37,270] Levofloxacin, the L-isomer of ofloxacin, is the least active of newer fluoroquinolones against methicillin-sensitive *S. aureus* with an MIC_{90} range of 0.25 to 0.5 mg/L but still significantly more active than ciprofloxacin.[36] Trovafloxacin, moxifloxacin, and gatifloxacin all have shown activity equivalent or superior to levofloxacin with MIC_{90} ranging from 0.06 mg/L to 0.5 mg/L.[36,37,274]

Early experimental studies showed that fluoroquinolone monotherapy was curative for many cases of staphylococcal infections involving orthopedic implants and that it was also effective in MSSA and MRSA endocarditis. Subsequent treatment failures were related to emergence of antibiotic-resistant isolates during or after therapy. However, these studies suggested that a combination of rifampicin and fluoroquinolone prevented emergence of resistance. Furthermore, combination therapy with a fluoroquinolone (fleroxacin plus rifampicin or fleroxacin plus rifampicin plus vancomycin) was observed to be highly effective and superior to single drugs alone (fleroxacin or vancomycin) in treatment of chronic staphylococcal foreign-body infections in a rat model.[277] Comparative clinical trials are few but they have also demonstrated efficacy of oral fluoroquinolones combined with either rifampicin or fucidic acid in staphylococcal infections.[273] For severe MSSA infections, combination of a fluoroquinolone and rifampicin has provided a clinical cure without decreased mortality in right-sided endocarditis,[278-280] in chronic osteomyelitis or in foreign body infections,[281,282] and in other deep-seated abscesses.[280]

After the introduction of the newer fluoroquinolones, they were considered as an alternative therapy to treat MRSA infections in particular which has, however, been complicated by rapid emergence of resistance.[283] Up to 80% of MRSA strains have become resistant to ciprofloxacin in Europe and the United States,[95,284-286] whereas most of the MSSA strains are susceptible to

fluoroquinolones.[287,288] The reasons for disparity in rates of fluoroquinolone resistance between MSSA and MRSA strains are uncertain. Cross-resistance among fluoroquinolones seems to be extremely common,[267,289] although the newer agents such as trovafloxacin and gatifloxacin may be active against these resistant strains.[24,283] Furthermore, fluoroquinolone use has been reported as an ecologic risk factor for high MRSA prevalence among hospitalized patients, and persistent colonization with MRSA.[287,290,291] Thus, fluoroquinolones do not offer in general a therapeutic alternative for the treatment of MRSA infections.[292]

2.6.4. Recommendations for antibiotic therapy in endocarditis

Left-sided native valve endocarditis

The most recent guidelines recommend a combination of an aminoglycoside with a beta-lactam or vancomycin for the first three to five days of treatment for left-sided native valve endocarditis.[22,27,28,76] Aminoglycoside should be administrated in a 3-times-daily dosing regimen, with a total daily dose not to exceed 3 mg/kg in patients with normal renal function.[27,293] Routine use of rifampicin has not been suggested for the treatment of uncomplicated left-sided native valve endocarditis in SAB.[22,76,126,137,157] However, rifampicin is recommended as an additive therapy in those patients who do not respond adequately to conventional treatment or have complicated endocarditis (e.g., myocardial or extracardiac deep infections).[23,27,35,164,239] There are no prospective, randomized, controlled studies to demonstrate the most appropriate duration of standard antistaphylococcal therapy.[62] Recommendations for treatment duration are largely derived from retrospective studies, consensus opinion or previously published recommendations. Thus, for patients with uncomplicated left-sided native valve IE, four to six weeks of beta-lactam or vancomycin treatment is sufficient.[22,27,126] For patients with complicated endocarditis, six weeks of standard antibiotic therapy should be used.[27]

Prosthetic valve endocarditis

Staphylococcal infections of prosthetic heart valves due to MSSA or MRSA are recommended to be treated with three antibiotics in combination. An aminoglycoside is initiated together with a beta-lactam or vancomycin for the first two weeks of therapy.[22,27,76,126] If the strain is resistant to all aminoglycosides, a fluoroquinolone (such as moxifloxacin or gatifloxacin) to which it is susceptible may be used instead of an aminoglycoside.[157,177,277,294] However, it should be noted that there is no clinical data to support this recommendation. Rifampicin is combined with a standard antibiotic for at least six weeks course of therapy in prosthetic valve endocarditis.[22,23,126,164,295]

Right-sided endocarditis

Right-sided endocarditis predominates among IDUs and involves a different pathophysiology. It is easier to cure and it can heal spontaneously in experimental models.[86,226,296] Several clinical studies have demonstrated that uncomplicated cases of right-sided endocarditis may be treated successfully with only 2-week regimens of a semisynthetic penicillin and aminoglycoside to reduce the expense and inconvenience of the four weeks duration of therapy.[176,257,297-299] A short-course therapy with glycopeptides (vancomycin or teicoplanin) and an aminoglycoside is associated with a high rate of clinical and microbiological failure and should not be used.[300,301] The standard 4-week therapy for right-sided endocarditis is recommended in situations of (1) a slow clinical or microbiologic response >96 h after initiation of antibiotic therapy; (2) complicated right-sided endocarditis with extracardiac deep infections, heart failure, or valve vegetations >2 cm; (3) right-sided endocarditis caused by MRSA; and (4) severe immunosuppression or AIDS.[86,186,191,302]

Uncomplicated right-sided endocarditis among IDUs have been treated successfully with oral antibiotic treatment alone.[278,279] Heldman and colleagues demonstrated[279] that a combination of oral ciprofloxacin and rifampicin given for four weeks was as effective as a 4-week regimen with parenteral oxacillin plus aminoglycoside. This oral regimen may be a reasonable alternative for those IDUs who are unwilling or unable to receive intravenous therapy.[62,186]

2.6.5. Recommendations for antibiotic therapy in other deep infections

In clinical practice, rifampicin is often suggested to be used in combination with standard antibiotic therapy in deep-seated abscesses,[32,280] osteomyelitis,[35,269] foreign body infections,[303,304] or because of poor response to the standard treatment in SAB.[33,34] The optimum duration of a parenteral beta-lactam or vancomycin for invasive (e.g., osteomyelitis or deep-seated abscesses) and orthopedic implant-related *S. aureus* infections is controversial, but it is suggested that they should continue for four to six weeks.[23,86,273,305] More recently short term parenteral therapy followed by prolonged (three to six months) oral therapy with rifampicin plus a fluoroquinolone has been proven to be effective in osteomyelitis and foreign body infections.[146,282,304,306,307] If conservative treatment fails or removal of infected foreign body material is impossible (e.g., due to technical difficulties or severe underlying diseases) lifelong antimicrobial treatment is needed and may prevent progression of the infection for many years.[214]

2.6.6. Surgical treatment

Infected foreign bodies in SAB should be removed whenever possible. For patients with an eradicable focus, both the mortality and the recurrence rates have been observed significantly lower as compared to those patients, in which the focus was not eradicated.[8] Surgical treatment of patients with infected orthopedic devices include debridement with retention of the prosthesis, one- or two-stage exchange, resection arthroplasty, arthrodesis, and amputation.[213,303] The use of antimicrobial-impregnated cement is suggested.[308] In acute osteomyelitis that is unresponsive to antimicrobial therapy, surgical decompression may be required.[309] Successful surgical therapy in chronic osteomyelitis includes debridement of the infected bone and soft tissues and revascularization of a poorly perfused region.[309] Most abscesses and empyemas require drainage. However, there is limited evidence available that some small abscesses in clinically stable patients respond to medical therapy without drainage.[120,146]

Several studies suggest that patients with staphylococcal endocarditis should be considered for valve replacement as an adjunctive therapy because of improved outcome.[157,310] The generally accepted indications for surgical intervention in endocarditis include congestive heart failure, uncontrolled infection, more than one serious systemic embolization, hemodynamically significant valvular dysfunction, or local suppurative complications such as perivalvular or myocardial abscesses.[76,164] Surgical treatment is required in up to 45% of patients with left-sided native valve endocarditis.[18,169] Because of the high mortality associated with *S. aureus* prosthetic valve IE, early surgical replacement is almost always recommended during concomitant antibiotic therapy.[174,311,312]

Enthuasism for cardiac surgery varies for patients with drug addiction, because there is lack of controlled trials upon which to base decision making.[302] Furthermore, the surgical approach is more conservative among IDUs due to continued use of injecting drugs and higher incidence of recurrent IE compared with the general population.[186] Cardiac surgery was observed necessary in only small minority of narcotic addicts[185] although other studies confirmed that surgical treatment clearly improved their survival as well.[313] However, indications for valve replacement in left-sided endocarditis among IDUs are the same as in the general population. Persistent infection is the indication for surgery in over 70% of right-sided endocarditis.[164] Tricuspid valvectomy or vegetectomy with valvuloplasty are the recommended surgical interventions for refractory right-sided involvement.[314,315]

2.7. Bacterial strain characteristics and host serological responses in *Staphylococcus aureus* infections

2.7.1. Bacterial virulence factors

Components of the cell envelope

Staphylococcal infections are characterized by penetration by the bacteria from the bloodstream into tissues resulting in dissemination, abscess formation and metastatic infection. *S. aureus* is also capable of evasion of phagocytosis by neutrophils.[316] Tissue invasion and killing by phagocytes are involved in the inflammatory response that leads to septic shock.[317] The virulence of *S. aureus* depends upon the effectiveness of the host defence in recognizing and dealing with components of cell wall, expression of a capsule and/or slime layer, and a wide variety of bacterial extracellular toxins and enzymes which are important both for the invasiveness as well as persistence of bacteria in infected organs.[318-320]

The staphylococcal cell wall consists of peptidoglycan, teichoic acid and various proteins, called adhesins (**Fig. 3**). Characteristic features of the peptidoglycans are endotoxin-like activity, stimulation of macrophages to release cytokines, activation of the complement cascade and aggregation of platelets.[28,321] Teichoic acids are likely to serve as bacteriophage receptor sites for attachment of cell-wall active enzymes and other proteins, and have recently been shown to play a key role for adherence of *S. aureus* into nasal epithelium.[86,322] Teichoic acids also activate the alternative complement pathway and the lectin pathway. Lipoteichoic acids are the plasma membrane-bound counterparts of teichoic acids. They have been observed to initiate inflammation by triggering the release of cytokines by macrophages.
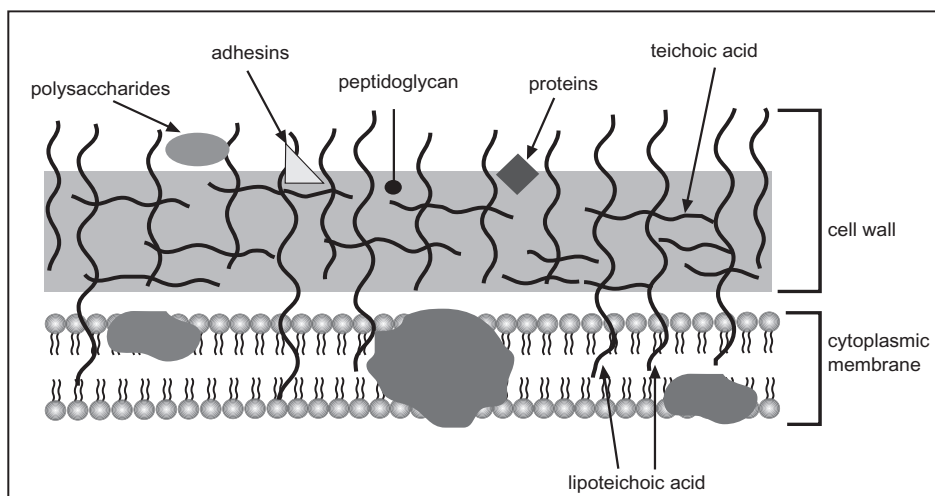
Figure 3. Structure of the cell envelope including the cell wall and cell membrane. Modified from the figure by Vidar Bakken.[323]

Adhesion and invasion

Staphylococci can produce several adhesins or microbial surface components recognizing adhesive matrix molecules (MSCRAMMs),[28] which are known to mediate staphylococcal adhesion to various cell surface proteins like fibronectin, fibrinogen, collagens, vitronectin, laminin, thrombospondin, bone sialoprotein and elastin.[324] The effects of binding of these proteins enable staphylococci to colonize tissues and initiate infection in wounds, joint cartilage, bones and heart valves.[28,325,326] Recent obervations on the cabability of *S. aureus* to invade eukaryotic cells have provided new information why antibiotic treatment of staphylococcal infection in many cases fails or reactivates due to the fact that the drug given can not reach intracellular bacteria.[327] It has been demonstrated that *S. aureus* uses fibronectin-binding proteins (FnBP) on its surface for invasion into mammalian cells and $\alpha_5\beta_1$-integrin on the surface of the host cell.[328]

The slime layer is an extracapsular, polysaccharide structure, which may influence virulence by increasing bacterial adhesion to endothelial cells and inhibiting phagocytosis.[319] Serotype 5 or 8 capsular polysaccharides compose the majority of all isolates obtained from cases of SAB.[28] Staphylococci have a special tendency to adhere to the polymer surface of plastic material, develop microcolonies and produce an extracellular polysaccharide (glycocalyx or slime) which forms a biofilm covering the microorganism.[329] The biofilm protects the bacteria from phagocytosis and the action of antibiotics.

Extracellular enzymes and toxins

S. aureus produces and secretes a number of extracellular enzymes (e.g., staphylokinase, protease, coagulase, hyaluronidase and lipase) and toxins (e.g., haemolysins, Panton-Valentine leukocidin, enterotoxins, toxic shock syndrome toxin-1 and epidermolytic toxins) which have been implicated as potential virulence factors.[86,319] Staphylokinase (SAK) is a plasminogen-activator protein and is responsible for the fibrinolytic activity of *S. aureus* which has an important role in disseminated intravascular coagulation (DIC) in sepsis.[319,330] Staphylokinase-plasminogen complex may also affect bacterial invasion into the host tissues.[331,332] SAK production has been connected to uncomplicated bacteremia and better prognosis.[333] Additionally, expression of SAK may be a requirement for the persistence of staphylococcal nasal carriage.[330] These findings suggest that SAK is one part of the adaptive mechanisms of *S. aureus* which favours bacterial symbiosis with the host.

S. aureus has a minimum of four haemolysins of which α-haemolysin is best characterized and most potent membrane-damaging toxin. *S. aureus* α-haemolysin is known to induce apoptosis in various cell types including keratinocytes, endothelial (e.g., heart valves) and epithelial cells at low concentrations.[334] *S. aureus* produces also three type of proteases which all may be responsible, either directly or indirectly, for connective tissue damage.[319]

Panton-Valentine leukocidin (PVL) is a cytotoxin, which exhibits highly specific lytic activity on human polymorphonuclear cells and monocytes.[335] PVL is a bicomponent toxin that consists of the polypeptides lukS-PV and lukF-PV.[336] It releases proinflammatory mediators and vasodilatating factors that induce severe inflammatory lesions,[337] and causes leukocyte destruction and tissue necrosis.[209] PVL is detected in fewer than 5% of all *S. aureus* clinical isolates. It is especially found in community-acquired MRSA but also in MSSA strains.[338] it is mainly associated with necrotic lesions of skin and subcutaneous tissues, such as furuncles, and also with severe, necrotizing, community-acquired pneumonia.[209,339] Although PVL-producing *S. aureus* affects healthy children and young adults the mortality rate of necrotizing pneumonia is nearly 75%.[94,340]

Staphylococcal enterotoxins, toxic shock syndrome toxin-1, and epidermolytic toxins display superantigenic properties.[341,342] Superantigens cause endotoxin-like shock including endothelial leakage, hemodynamic shock, and multiorgan failure by releasing proinflammatory cytokines.[86,319] Staphylococcal superantigens may also be important virulence determinants in experimental septic arthritis.[343]

2.7.2. Molecular typing and clonal spread of *Staphylococcus aureus*

Typing of *S. aureus* is mainly indicated in hospital outbreaks and for characterization of methicillin-resistant strains. During the last decades, traditional methods of *S. aureus* strain typing, such as serotyping and bacteriophage typing, have been supplemented and replaced with newer molecular methods including plasmid fingerprinting, ribotyping, PCR-based methods, sequencing and pulsed-field gel electrophoresis (PFGE).[323,344] Bacteriophage typing can still, in some cases, be considered for epidemiologic studies when a large collection of *S. aureus* strains from different geographical areas and laboratories is to be typed.[344] At present, the golden standard typing method for *S. aureus* is PFGE, where the staphylococcal genomic DNA is cleaved into large DNA pieces by the restriction enzyme *SmaI* followed by pulsed gel electrophoresis in order to obtain sufficient separation of the DNA pieces.[345] Furthermore, multilocus sequence typing (MLST) is based upon DNA sequencing of seven housekeeping genes and provides a method to identify clonality of MRSA or MSSA isolates.[346] Resistance to methicillin is determined by the *mec*A gene, which is a part of an additional DNA region, the staphylococcal cassette chromosome *mec* (SCC*mec*).[87] At least five SSC*mec* types have been identified (I-V).[338,347] Types I-III are shown to belong to hospital clones, whereas types IV and V of SCC*mec* are associated with community-acquired MRSA infections.[86]

Molecular typing techniques have not only allowed the identification of pandemic clones of MRSA, but have also enabled the monitoring of MRSA clones circulating in different hospitals and at different time intervals.[348] Five major MRSA clones (i.e., Iberian, Brazilian, Hungarian, New York-Japan and Paediatric pandemic clones) account for almost 70% of isolates that have spread in recent years across the continents.[348] Cross infections and epidemic spread of a single *S. aureus* (including MRSA) clone in addicts have been reported in Europe and North America.[182,349,350]

In Finland, the majority of clinical MSSA isolates (from all infection sites) share genotypes with non-multiresistant MRSA, including community-acquired MRSA.[87] In agreement with another recent Finnish report,[351] dissemination of MRSA is not entirely due to clonal spread of multiresistant pandemic clones. Instead, several prevalent clones of MSSA seem to have acquired the SCC*mec*. Epidemic MRSA strains FIN-16 and FIN-21 are shown to be associated most frequently with MRSA bacteremias in Finland.[352] Furthermore, FIN-4, FIN-11 and FIN-14 strains have been related to community-acquired MRSA infections.[353]

The highly virulent community-acquired MRSA strain USA400 (prototype strain, MW2) with PVL production has been observed to cause fatal septicaemia and septic arthritis among healthy children and young adults in the United States.[354,355] It is unclear whether MSSA clones have a particular ability to cause serious infections. In a recent study, MSSA clones with sequence types (ST) 1, 25, and 30 were associated with community-acquired invasive disease, which may indicate virulence of these clones.[346]

2.7.3. Serological diagnostic assays

Teichoic acid antibody

During the past 30 years numerous serological tests have been introduced to distinguish between patients with uncomplicated or complicated SAB. The teichoic acid antibody (TAA) assay is the most thoroughly investigated serological test and probably also the most frequently used. A TAA titer of 1:8 or more is considered clearly positive in patients with SAB, especially in endocarditis.[69,356] A titer of 1:2 or 1:4 should be regarded as suggestive for a *S. aureus* infection although cross-reactions with other bacteria have been reported.[357] In addition, a generally acceptable sign of active *S. aureus* disease is a fourfold rise in TAA titer.[356] A TAA response is expected to develop during the first 14 to 28 days of infection.[358,359] However, almost one third of patients may not have detectable antibodies on admission to hospital.

During the 1970s and 1980s several studies on TAA determined by gel diffusion and counter-immunoelectrophoresis were published, until the more sensitive and specific gel diffusion assay (Endo-Staph) became available.[360] Results from the different studies have shown extreme variability due alterations of standardization.[360,361] For example, patients with complicated SAB had a positive TAA response in between 23% to 100%,[318,358,362-364] while in some studies even 44% of healthy control sera were positive.[363] Several possible explanations for the divergent results of serologic studies have been proposed: (1) variation of antigen preparation; (2) differences in methods used; (3) variation in the cut-off points for positive serological response; (4) limited patient material; (5) varying criteria for selecting patients; (6) and lack of accurate definitions for the endocarditis.[360,365] The highest sensitivity for elevated TAA values has been seen in patients with *S. aureus* endocarditis, especially among IDUs.[356,357,359,366-368] This serological response in IDUs is most probably due to previous and recurrent intravenous exposure to *S. aureus*.[362,368] Furthermore, use of TAA assay may be useful particularly in chronic *S. aureus* infections.[369]

Antibody against staphylolysin (α-haemolysin)

The first test available for routine use was the antistaphylolysin (ASTA) assay. A titer of ≥2.0 IU/ml in it has been regarded as positive.[370] The diagnostic value of ASTA is considered limited due to its low sensitivity.[356] This has been shown in some studies, where the serological response was observed in only 32% to 62% of patients with complicated SAB or endocarditis.[371,372] ASTA has been extensively studied in dermatological patients where ASTA values have correlated to the barrier function of the skin rather than to the actual stage of infection.[373] High ASTA titers have been found in patients with various dermatoses, especially those with atopic dermatitis and chronic pruritic dermatoses.[373,374] Comparisons of TAA and ASTA have shown correlation between antibodies against these two antigens with TAA being positive earlier than the ASTA.[356,375] However, no single serologic assay has proven to be positive in all patients with SAB or to be able to differentiate between patients with uncomplicated or complicated bacteremia.[360]

Combined serological tests

Various *S. aureus* antigen preparations including whole *S. aureus* cells, peptidoglycan, teichoic acid, α-haemolysin, an ultrasonicate of *S. aureus* cells and lipase have been used.[365,369] The combined use of these various serological tests may increase their positive predictive value.[320] However, according to recently published data none of the assay combinations have become valuable diagnostic aids in SAB or endocarditis.[365] In addition, antibody levels against teichoic acid, α-haemolysin and lipase were even lower in patients with complicated SAB as compared to those with uncomplicated SAB in one study.[376] The need for serological tests to assist in the diagnosis of *S. aureus* infections has changed in favour of other techniques such as computed tomography, isotope scanning, echocardiocraphy, and magnetic resonance.[320]

3. AIMS OF THE STUDY

The purpose of the present study was to evaluate the epidemiology, incidence, clinical manifestations, treatment, outcome, strain characteristics and their virulence factors, and host immune responses among patients with SAB in a country with low prevalence of methicillin resistance.

The specific aims were:

I   To evaluate trends in the incidence, outcome and morbidity of SAB in Finland during 1995-2001 and to assess the proportion of nosocomial versus community-acquired SAB.

II   To find out if levofloxacin combined with the currently used antibiotic treatment of SAB would improve the patient outcome as reduced mortality or complications such as deep infections.

III To compare risk factors, site of valvular involvement, clinical manifestations, and outcome of *S. aureus* endocarditis among IDUs and nonaddicts.

IV   To compare patient characteristics, the bacterial strains and their virulence factors, and host immune responses in order to explain the different prognosis and risk of developing endocarditis among IDUs and nonaddicts.

4. MATERIALS AND METHODS

4.1. Patients

Study I was based on a retrospective laboratory-based surveillance data on *S. aureus* bacteremia (SAB) in Finland. From January 1995 to December 2001, at least one isolation of *S. aureus* from blood was informed by 30 clinical microbiology laboratories to the National Infectious Disease Register (NIDR). Each notification included the following information: date of specimen, patient`s date of birth, patient`s sex and treating healthcare facility. Using this information and a time interval of three months, multiple episodes of the same case were merged into one case, either by the notifying laboratory or in the NIDR database (*n* = 5690 notifications). A case was defined as a person with SAB identified through the NIDR from 1995 to 2001. National identity codes for each person with SAB were collected retrospectively from the primary diagnostic laboratory, either in electronic format or on paper. After the collection of the national identity codes from each notified patient, and after exclusion of recurrent episodes, a total of 5045 cases were identified.

Studies II-IV comprised of a prospective, randomized, multicenter trial conducted in five university hospitals and seven tertiary care hospitals in Finland. Adult patients with at least one blood culture positive for methicillin-sensitive *S. aureus* (MSSA) were included within 1-7 days of blood culture sampling. The first part of the study included patients from January 1999 to May 1999 (designated as FINTROVA) but was interrupted due to withdrawal of the study drug, trovafloxacin, from the market. The study was continued using levofloxacin with the same patient group from January 2000 to August 2002 (designated as FINLEVO). Patients with SAB were randomly assigned to receive either standard treatment or standard treatment combined with trovafloxacin (FINTROVA) or levofloxacin (FINLEVO). Randomization was done blindly and separately at each study location after the patient or his/her representative had given written informed consent. However, seriously ill patients, for example unconscious patients with assisted ventilation, could be taken into the study without signed informed consent, because they were assumed to benefit most from the study medication. As soon as possible, a signed informed consent was taken from the patient or his/her representative. After randomization the treatments were open for the investigator and the patient.

Exclusion criteria included age younger than 18 years, imprisonment, proven or suspected pregnancy, breastfeeding, epilepsy, another bacteremia during the previous 28 days, polymicrobial

bacteremia (≥3 microbes), history of allergy to any quinolone antibiotic, previous tendinitis during fluoroquinolone therapy, prior fluoroquinolone use for more than five days before randomization, positive culture for *S. aureus* only from a central intravenous catheter, neutropenia (<0.5 x 10^9/L), failure to supply an informed consent or glucose-6-phosphate dehydrogenase deficiency (FINTROVA only). Patients with bacteremia due to MRSA and a *S. aureus* strain resistant to any fluoroquinolone and those with meningitis at the time of randomization were also excluded. In total, 430 patients were randomized into both trials (49 patients into FINTROVA and 381 into FINLEVO).

4.2. Study designs

Study I was a retrospective epidemiological population-based study, in which the trends in the age- and sex-specific annual incidence, proportion of nosocomial versus community-acquired bacteremia, and outcome of SAB during 1995-2001 were evaluated. Preceding hospitalizations for all study patients with SAB were obtained from the national hospital discharge registry (HILMO), which included records on patient`s identity information, admission and discharge dates, healthcare provider, type of service, specialty, patient`s place of residence (home or institution) at the time of presentation to the institution, and data on surgical procedures. The outcome (case fatality rate) at seven days and 28 days, and at three months after the date of the first *S. aureus*-positive specimen for a particular patient was obtained from the national population registry, using the national identity codes.

Study II was a prospective trial, in which 1226 patients with SAB were identified during the FINLEVO study period (**Fig. 4**). In total, 381 patients were included in the analysis, with 191 patients in the levofloxacin and 190 patients in the standard treatment group. All patients were followed up by an infectious disease specialist during the hospital treatment and thereafter with control visits at 28 days and at three months. Primary end-points were case fatality rate at 28 days and at three months. Secondary outcome measures included the number of complications (e.g., deep infections) observed after the first week of antibiotic treatment, decrease in serum C-reactive protein (CRP) concentration, length of antibiotic treatment, need for surgical intervention, and time to defervescence. Laboratory tests were conducted on the day of positive blood culture for *S. aureus*, at randomization and every other day during the first week, twice a week thereafter during hospitalization, at 28 days, and at three months.

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                    ┌─────────────────────────────┐
                    │      1226 patients with     │
                    │ Staphylococcus aureus       │
                    │         bacteremia          │
                    └─────────────────────────────┘

┌─────────────────────────┐
│      846 patients        │
│ excluded or not evaluated*│
└─────────────────────────┘

                    ┌─────────────────────────────┐
                    │      381 randomized in       │
                    │  intention-to-treat analysis │
                    └─────────────────────────────┘

        ┌──────────────────────┐      ┌──────────────────────┐
        │ 191 in levofloxacin  │      │  190 in standard     │
        │       group          │      │   treatment group    │
        └──────────────────────┘      └──────────────────────┘

┌──────────────────────────┐          ┌──────────────────────────────┐
│ 19 patients excluded      │          │ 11 patients excluded          │
│ (levofloxacin <2 wk)      │          │ (any fluoroquinolone ≥1 wk)   │
└──────────────────────────┘          └──────────────────────────────┘

                    ┌─────────────────────────────┐
                    │      351 patients in         │
                    │    per-protocol analysis     │
                    └─────────────────────────────┘

        ┌──────────────────────┐      ┌──────────────────────┐
        │ 172 in levofloxacin  │      │  179 in standard     │
        │       group          │      │   treatment group    │
        └──────────────────────┘      └──────────────────────┘
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Figure 4. Patients with *Staphylococcus aureus* bacteremia in the study sites for FINLEVO and number of patients incluced into analyses. *Excluded patients consisted of 100 patients with failure to supply an informed consent or patient refusal, 48 with neutropenia ($<0.5 \times 10^9$/L), 42 deaths prior to randomization, 30 with epilepsy or prior convulsion, 23 with prior fluoroquinolone use for more than 5 days preceding randomization, 15 with a fluoroquinolone resistant strain of *S. aureus*, 15 with meningitis, 13 with polymicrobial bacteremia (≥3 microbes), eight with a positive culture for *S. aureus* only from a central intravenous catheter, seven with another bacteremia during the previous 28 days, five with bacteremia due to methicillin-resistant *S. aureus*, three with proven or suspected pregnancy, two prisoners, two breastfeeding women, one with a history of allergy to quinolone, one with bacteremia caused by borderline oxacillin-resistant *S. aureus*. Additionally, 530 patients with SAB were identified during the study period but not evaluated for the trial.

Study III comprised all patients with endocarditis from the original FINTROVA and FINLEVO trials of 430 patients with SAB. Definite or possible endocarditis as defined by the modified Duke criteria[75] was found in 74 patients, of whom 20 were IDUs and 54 were nonaddicts. Transthoracic echocardiography (TTE) and/or transesophageal echocardiography (TEE) was performed as clinically indicated by experienced echocardiographers. Two-dimensional imaging from multiple tomographic planes and spectral Doppler and colour flow imaging were used in all study sites. The presence of cardiac vegetations, oscillation, paravalvular or intracardiac abscess, new valvular regurgitation, prosthetic valve dehiscence, and valve perforation were recorded.[75] Case fatality rates were obtained at seven days and 28 days, and at three months. Other clinical outcome measures were the site of valvular involvement, evidence of extracardiac deep infections or thromboembolic events, need for cardiac surgery, and duration of fever and hospitalization.

Study IV comprised all patients with identifiable IDUs and an equal number of controls from the original FINTROVA and FINLEVO trials of 430 patients with SAB. In total, 44 IDUs were identified and 20 of them had endocarditis (19 definite according to modified Duke criteria).[75] To study differences in clinical characteristics, bacterial strains and host serological responses between IDUs and nonaddicts, a control patient for each addict was chosen. For each case with endocarditis (n=20), we chose a control with preferably definite endocarditis and obtaining a convalescent serum sample at 28 days. Thus, controls with endocarditis consisted of 16 definite and four possible endocarditis. For each case without endocarditis (n=24), we chose an age (±15 years) and sex matched control whose randomization time was the nearest possible. The site of valvular involvement, evidence of deep infections or thromboembolic events, and duration of fever and hospitalization were detected within three months follow-up. *S. aureus* isolates were genotyped by pulsed-field gel electrophoresis (PFGE), and tested for Panton-Valentine leukocidin (PVL), staphylokinase (SAK), protease and haemolysin production. Acute and convalescent sera were tested for antibodies against α-haemolysin (ASTA) and teichoic acid (TAA). Information on drug injection history, drug-use practices, and drugs were available only from 29 IDUs.

4.3. Definitions

In Study I, SAB was defined as nosocomial if the first positive blood culture was obtained two days or more after hospital admission or if obtained within two days of admission with a preceding hospital discharge within seven days. SAB was classified as community-acquired if the specimen positive for the blood culture was obtained within two days of admission and there

were no hospitalizations within the preceding seven days. The origin of SAB was defined as unknown if no periods of hospitalization could be identified for a patient.

In studies II-IV, SAB was hospital-acquired if the first positive blood culture was obtained ≥48 hours after admission, or the patient was a resident in a long-term care facility or attended hemodialysis within the preceding two months. All other cases were defined as community-acquired. Prognosis or severity of underlying diseases were divided as healthy, nonfatal, ultimately, or rapidly fatal according to the criteria of McCabe and Jackson.[377] The infection focus was classified as definite if it was documented by bacteriological, radiological or pathological investigations, but suspected if it was evident from clinical findings only. Infection of a central intravenous catheter was defined by the guidelines of the Infectious Diseases Society of America.[133] Endocarditis was classified as definite or possible using the modified Duke criteria by clinical, pathological, and echocardiographic data.[75] Deep infection was characterized as endocarditis, pneumonia, deep-seated abscess, osteomyelitis, septic arthritis, meningitis, septic thrombophlebitis, mediastinitis, urinary tract infection, infection of any prosthetic device or recurrent SAB. Relapse of SAB was confirmed by the same resistance pattern and PFGE typing for two *S. aureus* strains. Other recurrences of *S. aureus* culture in the blood were classified as reinfections. CRP was defined as normal when less than 10 mg/L. Leukocytosis was determined if white blood cell count was over 12×10^9/L and leukopenia when white blood cell count was less than 4×10^9/L. Thrombocytopenia was defined as a blood platelet count less than 100×10^9/L, and acidosis with venous blood pH <7.30. Elevated liver enzymes were defined as an increase of alanine aminotransferase (ALT) >120 U/L in females and >150 U/L in males, alkaline phospatase >300 U/L or bilirubin >40 µmol/l. Time to defervescence was recorded in days until the axillary temperature was <37.5°C.

In studies III-IV, IDUs were defined as patients who had injected drugs within the past six months before randomization. Severe sepsis at the time of the first blood culture positive for *S. aureus* was classified as an infectious process leading to organ dysfunction or signs of hypoperfusion or hypotension.[378] Furthermore, endocarditis of a prosthetic valve was defined as early when occurring ≤60 days after valve replacement and as late >60 days after valve replacement. Arterial thromboembolic events comprised acute myocardial infarction or unstable angina, parenchymal and cerebral embolization or infarction, and mycotic aneurysm. Venous thromboembolic manifestations were defined as septic or venous pulmonary embolism, and deep venous thrombosis.

4.4. Study treatments

Studies II-IV consisted of patients with SAB who were randomly assigned to receive either standard treatment or it combined with a fluoroquinolone (trovafloxacin or levofloxacin). However, Study II presents only the effect of levofloxacin combination therapy on patient outcome. The results of trovafloxacin efficacy in combination therapy have not been analyzed or published.

The study protocol for antibiotic treatment in Study II is summarized in **Table 1**. Primarily, the standard treatment consisted of an intravenous semisynthetic penicillin, cloxacillin or dicloxacillin (2 g q4h). Alternatively, cefuroxime (1.5 g q6h), clindamycin (600 mg q6-8h), or vancomycin (1 g bid) were allowed if a contraindication for the use of penicillins was noted. However, clindamycin was not recommended in endocarditis. When oral treatment was indicated, cloxacillin (500 mg q6h), cephalexin or cefadroxil (500 mg q6h), or clindamycin (300 mg q6h) were accepted as standard therapy. In cases of renal dysfunction the antibiotic doses were adjusted as recommended by the manufacturers. In the fluoroquinolone treatment group, the dose of levofloxacin both intravenously and orally was 500 mg once daily for patients under 60 kg and 500 mg bid for those over 60 kg in weight. If endocarditis was clinically suspected or confirmed, an aminoglycoside (either tobramycin or netilmicin at 1 mg per kilogram of body weight q8h) was added to the drug therapy described above. Rifampicin (450 mg once daily for patients under 50 kg and 600 mg once daily for patients over 50 kg in weight, orally or intravenously) was given if there was a suspicion or evidence of endocarditis, or other deep infections. Any antibiotic treatment was avoided or discontinued if 1) a contraindication (e.g., renal failure and an aminoglycoside), 2) a serious adverse event such as an allergic reaction, or 3) a drug interaction occurred or could be expected (e.g., problematic warfarin therapy during rifampicin).

Table 1. Study protocol for antibiotic therapy in patients with *Staphylococcus aureus* bacteremia randomized either standard treatment or combined with levofloxacin.

STANDARD TREATMENT	STANDARD TREATMENT + LEVOFLOXACIN
1) Cloxacillin[a]	**1) Cloxacillin**[a] **+ levofloxacin**
2) Penicillin allergy Cefuroxime or Clindamycin or Vancomycin	**2) Penicillin allergy** Cefuroxime + levofloxacin or Clindamycin + levofloxacin or Vancomycin + levofloxacin
3) Endocarditis[b] Cloxacillin[a] + aminoglycoside + rifampicin	**3) Endocarditis**[b] Cloxacillin[a] + aminoglycoside + rifampicin + levofloxacin
4) Endocarditis with penicillin allergy[b] Cefuroxime + aminoglycoside + rifampicin or Vancomycin + aminoglycoside + rifampicin	**4) Endocarditis with penicillin allergy**[b] Cefuroxime + aminoglycoside + rifampicin + levofloxacin or Vancomycin + aminoglycoside + rifampicin + levofloxacin
5) Deep infection Cloxacillin[a] + rifampicin	**5) Deep infection** Cloxacillin[a] + rifampicin + levofloxacin

[a]Alternatively dicloxacillin.

[b]Includes both right-sided and left-sided endocarditis, and native and prosthetic valve involvement.

The duration of antibiotic treatment was determined by the treating physician. However, all patients received at least 14 days of intravenous antibiotic treatment. In SAB associated with a central intravenous catheter the antibiotic treatment was discontinued after 14 days when the catheter was removed, and there were no evidence of endocarditis or other deep infections (**Table 2**).[133] When endocarditis or other deep infection was verified or clinically suspected, intravenous standard antibiotic and rifampicin were recommended to be continued for at least four to six weeks. In endocarditis, aminoglycoside was completed after seven days.[22,28] In the fluoroquinolone treatment group, patients received levofloxacin for at least for four weeks of which the drug was given intravenously at least for the first 14 days.

Table 2. Duration of the standard antibiotic treatment in different subgroups of *Staphylococcus aureus* bacteremia.

Subgroups	Duration	Comments
Catheter-related SAB Cloxacillin[a]	2 wk iv	A 2-wk intravenous therapy is recommended, if the catheter has been removed, patient is afebrile, CRP is <10 mg/L, and there are no signs of endocarditis or other deep infections.
SAB without deep infection Cloxacillin[a]	2 wk iv + 2 wk po	After 2 wk intravenous therapy, oral therapy is administrated for another 2 wk, if patient is afebrile, CRP is <10 mg/L, and there are no signs of endocarditis or other deep infections.
Endocarditis Cloxacillin[a] Rifampicin Aminoglycoside	4 - 6 wk iv at least 4 wk 7 days	4 to 6 wk duration of intravenous semisynthetic penicillin + rifampicin is recommended in both right-sided and left-sided endocarditis. After intravenous administration, duration of oral antibiotic therapy is based on clinical judgment.
Deep infection Cloxacillin[a] Rifampicin	4 - 6 wk iv at least 4 wk	After 4 to 6 wk intravenous administration, duration of oral antibiotic therapy is based on clinical judgment.

[a]Alternatively dicloxacillin.

4.5. Microbiological methods

Blood was cultured using the BacT Alert System (Organon-Teknika, Boxtel, The Netherlands) in seven hospitals and the Bactec system (BD Diagnostic Systems, Sparks, Md, United States) in five hospitals. Aliquots of bottles with a positive signal were Gram stained and subcultured on chocolate agar plates. *S. aureus* isolates were identified by standard laboratory methods including colony morphology, Gram staining, production of DNAase and urease as well as ability to use mannitol and trehalose. Antimicrobial drug susceptibility was tested by the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The antibiotics tested included oxacillin, cephalexin, clindamycin, erythromycin, levofloxacin, trovafloxacin, fucidic acid, rifampicin, trimethoprim-sulfamethoxazole, and vancomycin. MICs of oxacillin were determined by E-test (AB Biodisk, Solna, Sweden) according to the manufacturer`s

instructions. The interpretative criteria for the zone diameters of growth inhibition were according to the CLSI with the following equivalent MIC breakpoints for levofloxacin: susceptible, ≤2 ug/mL; resistant, ≥8 ug/mL. For rifampicin, the used corresponding CLSI breakpoints were: susceptible, ≤1 ug/mL; resistant, ≥4 ug/mL.

In Study IV, PFGE was performed by using the Harmony protocol[379] where the genomic DNA was digested with the restriction endonuclease *SmaI* (Boehring Mannheim, Mannheim, Germany) in agarose blocks prepared as described elsewhere.[380] Chromosomal fragments were separated using a Chef DR III or Chef Mapper XA apparatus (Bio-Rad Laboratories, Hercules, CA). PFGE running conditions consisted of two blocks: block one with switching times of 5 s and 15 s and a running time of 10 h, and block two with switching times of 15 s and 60 s and a running time of 13 h. PFGE profiles were analyzed according to the following criteria: strains sharing identical band profile were considered the same, those differing 1-3 bands were interpreted as closely related, and those differing 4-6 bands were interpreted as possibly related. Strains differing for seven or more bands were considered to be unrelated.[344] According to these rules, strains differing by less than seven bands from one another were ascribed the same type name. The *S. aureus* PFGE database at the National Public Health Institute was used as the basis for naming the strains. Profiles that had been previously found among epidemic MRSA or MSSA strains were indicated by FIN- and MSSA-codes, respectively. A sporadic strain possessed a previously unknown PFGE profile.

Furthermore, in order to determine SAK production in Study IV, staphylococci were grown in Todd-Hewitt broth overnight at 37°C and centrifuged at 2700 x g for 10 min at room temperature. The clear culture supernatants were used for SAK determination as described.[332] Strains were also tested for haemolysin and protease production by streaking two colonies of a strain on bacterial agarose plates containing either sheep red blood cells (1 mg/ml) or skimmed milk powder (w/v). The haemolytic and protease activities were determined visually as negative if they were weaker than the ones caused by the control organism, *S. aureus* strain Cowan I, and positive if the effects were equal or stronger than the control. The presence of PVL genes (lukS-PV-lukF-PV) was detected by PCR.[209] To confirm the functionality of the PVL-PCR reaction and the quality of the DNA, *nuc* gene was amplified at the same time.[381] *S. aureus* strain CCUG 46923 was used as a positive control for the PCR.

4.6. Serological methods

In Study IV, serum samples were collected after the first positive blood culture for *S. aureus* at days two to seven (acute phase), and at days 22 to 28 (convalescent phase). After the samples had coagulated at room temperature, sera were collected by centrifugation and stored at -20°C for further analysis. Serum antibody titers against staphylolysin or staphylococcal α-haemolysin were measured as described by Larinkari with minor modifications.[373] Two-fold dilutions of heat inactivated (30 min at 56°C) patient sera, antistaphylolysin standard (Dade Behring Marburg GmbH, Marburg, Germany), and control sera were incubated with a determined amount of staphylolysin (Dade Behring) for 15 min at 37°C and, after addition of washed rabbit red blood cells, for an additional 45 min. After incubation, the red cells were mixed with a horizontal rotatory shaker, pelleted by centrifugation and finally incubated at room temperature for 3 h. Finally, the threshold for inhibition of haemolysis was determined as the first dilution where a yellowish colour was detectable around pelleted red blood cells. A single value ≥3.2 IU/ml, and a 4-fold or greater rise in titers were regarded as positive.

Antibody titers against staphylococcal ribitol teichoic acid were determined by a gel double diffusion assay according to manufacturer`s instructions (Endo-Staph[R], Meridian Bioscience Inc., Cincinnati, Ohio, USA). A single value ≥8, and a 4-fold or greater rise in titers were regarded as positive. The limits of ASTA and TAA titers represent 98-99th percentile values in the healthy Finnish population during the study period.

4.7. Statistical methods

4.7.1. Incidence rates

In Study I, annual data from the national population registry from years 1995-2001 were used as denominators to calculate age- and sex-specific incidence rates as well as the blood culture sampling rates. The average annual incidences during the surveillance period were calculated by using the total number of cases and population during 1995-2001. To evaluate secular trends, rates of SAB in different age and sex groups were calculated for each 12-month period from January 1995 to December 2001. Poisson regression with the PROC GENMOD procedure was used to assess whether the observed changes in the rates were statistically significant. Pearson correlation coefficients (and p values) were calculated over seven years for the relationship between the incidences of SAB and rates of blood-cultures processed.

4.7.2. Sample size and patient populations

In the sample size calculation, when mortality was assumed to be 10% in the levofloxacin group and 20% in the standard treatment group, a power of 80% would be achieved with 198 patients in each study arm (Study II). A two-tailed significance level of 5% was used. Data were analyzed from three different patient populations, primarily by intention-to-treat (ITT) analysis with 381 patients **(Fig. 4)**. Secondary analysis was performed by per-protocol (PP) (351 patients). Patients were ineligible for PP analysis if they had received levofloxacin <2 weeks in the levofloxacin group, or any fluoroquinolone for ≥1 week within the first 28 days after randomization in the standard treatment group. All collected data except demographic characteristics were analyzed in both ITT and PP populations, but only results from ITT analyses are shown. Additionally, the length of antibiotic therapy was analyzed from a population of which deceased patients were excluded (308 patients).

4.7.3. Statistical analysis

Statistical analyses were performed with Epi Info software, version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA) (Study I), SAS® version 8.2 (Study II), and SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) (Studies I, III and IV). The primary variable and other categorical variables were analyzed by the chi-squared test or Fisher`s exact test, as appropriate. Continuous baseline variables were compared using the t-test or the Mann-Whitney test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the significance of differences in patient groups.

In Study II, the stratified Cochran-Mantel-Haenszel (CMH) test was used in order to adjust for levofloxacin as a confounding factor when the effect of rifampicin was analyzed, and to adjust for other variables, including age, nosocomial acquisition, McCabe`s classification, endocarditis, and the number of deep infections to compare mortality between patients with or without rifampicin treatment. The results of variables described above have not been published before. Additionally, a decrease in serum CRP concentration was analyzed using Analysis of Variance for Repeated measurements (RMANOVA). Mortality and time to defervescence survival estimates were calculated with the Kaplan-Meier method. The log-rank test (Study II) and Cox regression analysis (Study III) were used to compare the survival estimates. Survival was calculated from the day of randomization (Study II) and from the day of the first positive blood culture (Study III) until three months. All tests were two-tailed, and $p < 0.05$ was considered to be significant.

4.8. Ethical aspects

Appropriate permissions were acquired from the Ministry of Social Affairs and Health, the Finnish data protection authority, and the National Research and Development Center for Welfare and Health to use the data from the national hospital discharge registry and the national population registry (Study I). FINTROVA and FINLEVO trials were approved by the ethics committees of all study sites and by the Finnish National Agency for Medicines (Studies II-IV). The patients or their representative gave a written informed consent. From seriously ill patients consent was taken as explained before (section 4.1.).

5. RESULTS

5.1. Trends and outcome of *Staphylococcus aureus* bacteremia in Finland during 1995-2001 (Study I)

5.1.1. Proportion and incidence of SAB according to hospital- and community-acquired cases, age, sex, and region

During the study period, 2546 of 5045 (51%) SAB cases were classified as hospital-acquired and 2203 (44%) cases as community-onset, while 296 (6%) were of unknown origin. Among the 2546 nosocomial SAB, the positive blood culture was obtained >2 days after hospital admission in 1794 (70%) cases and within two days in 752 (30%) cases. The proportion of nosocomial SAB was 51% in both 1995 and 2001 without any change during the observation period (range 48%-53% by year and 44-57% by region) (**Table 3**).

The median age of the patients was 62 years (range, 0-100) and 3041 (60%) were males. Female patients were significantly older than male patients (median age, 68 vs. 59 years; p <0.001), and the patients with nosocomial infection were significantly older than those with community-acquired infection (median age, 66 vs. 59 years; p <0.001). The incidence rates of SAB varied substantially by age and sex (**Table 4**) and were highest at the extremes of the life span. In all age groups, the incidence rates were at least 1.5 times higher in males than in females. The difference was most pronounced (i.e., 2.4-2.5 times higher) in the age groups between 35 and 64 years. In adult age groups, the rates consistently increased by age in both genders, beginning in males at clearly a younger age group than in females. The proportion of nosocomial SAB was highest in infants and elderly persons and was slightly higher in female than in males in age groups between 15 and 34 years.

Table 3. Incidence of *Staphylococcus aureus* bacteremia according to region and year, and proportion of hospital-onset infections, Finland, 1995-2001.

Region (population)	Incidence of SAB, cases per 100,000 population Proportion of hospital-onset infections (%)							
	1995	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**Overall**[a]
Helsinki (1,671,608)	10 (48)	10 (53)	15 (51)	13 (48)	15 (36)	16 (44)	16 (44)	14 (46)
Tampere (1,180,106)	11 (53)	13 (56)	13 (50)	14 (49)	13 (48)	14 (53)	14 (51)	13 (51)
Kuopio (869,943)	16 (50)	15 (58)	19 (58)	18 (51)	20 (62)	21 (60)	22 (55)	19 (56)
Oulu (728,680)	7 (64)	8 (52)	9 (45)	11 (61)	13 (56)	17 (57)	20 (59)	12 (57)
Turku (707,298)	11 (49)	12 (40)	13 (42)	9 (34)	15 (43)	11 (44)	12 (51)	12 (44)
All 5 regions (5,157,636)	**11 (51)**	**12 (53)**	**14 (51)**	**13 (49)**	**15 (48)**	**16 (51)**	**17 (51)**	**14 (51)**

SAB, *Staphylococcus aureus* bacteremia.
[a]Average annual incidence (cases per 100,000 population).

Table 4. Incidence of *Staphylococcus aureus* bacteremia by age group and sex, Finland, 1995-2001.

Age group (years)	Men		Women		Total	
	No. of cases	Rate[a]	No. of cases	Rate[a]	No. of cases	Rate[a]
<1	59	28	38	19	97	24
1-14	193	6	114	4	307	5
15-24	161	7	70	3	231	5
25-34	137	6	102	4	239	5
35-44	267	10	117	4	384	7
45-54	489	17	197	7	686	12
55-64	576	31	263	13	839	22
65-74	679	51	473	27	1152	38
>74	480	69	630	40	1110	49
All	**3041**	**17**	**2004**	**11**	**5045**	**14**

[a]Average annual incidence (cases per 100,000 population).

The average annual incidence of SAB was 14 cases per 100,000 population. The annual incidence of SAB rose by 55%, from 11 per 100,000 population in 1995 to 17 per 100,000 population in 2001 (p <0.001). The incidence of disease varied by region, being maximally from 14 cases to 22 per 100,000 population in 2001 (in Tampere and Kuopio, respectively) (**Table 3**). The increase in incidence was detected in both genders, although it was slightly greater in females (63%, from 8 per 100,000 population to 13) than in males (40%, from 15 per 100,000 population to 21). The increase occurred only in adults and was significant in all adult age groups

(p values from 0.012 to <0.001), except in the range of 55-64 years of age. The increase was most distinct in persons >74 years of age, in whom it rose from 36 per 100,000 population in 1995 to 63 per 100,000 population in 2001 (**Fig. 5**).
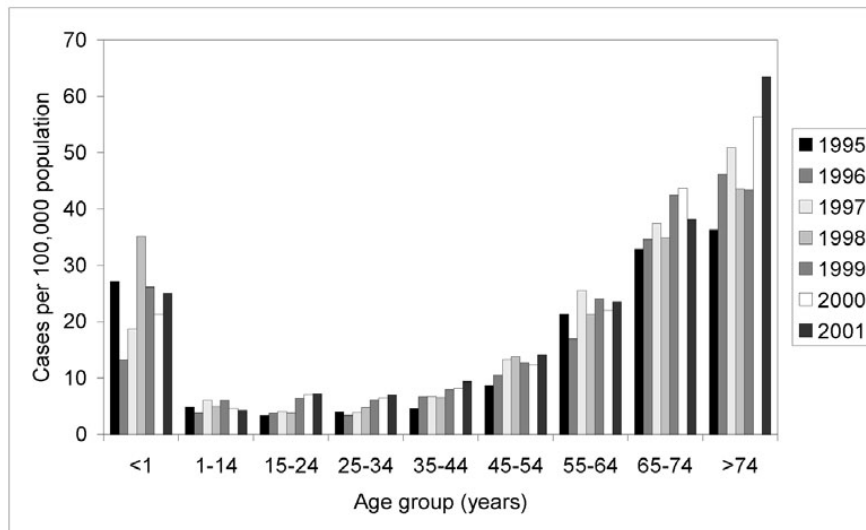

Figure 5. Annual incidence of *Staphylococcus aureus* bacteremia by age group, Finland, 1995-2001.

During the study period, a total of 1,128,321 blood culture sets were processed in 30 Finnish microbiology laboratories. The rate of blood culture sampling rose by 21%, from 28 blood-culture sets per 1000 population in 1995 to 35 in 2001 (range of increase by region, 9-27%), associated significantly with increasing incidence of SAB detected during 1995-2001 (p <0.001).

5.1.2. Outcome

Among the 5045 patients with SAB, 465 (9%) died within seven days after the first blood culture positive for *S. aureus,* 875 (17%) died within 28 days, and 1217 (24%) within three months. The median age of persons who died within 28 days was significantly higher than that of those who survived (73 vs. 59 years; p <0.001). The case fatality rate increased with age, being lowest in the age group 1-14 years and highest in the group >74 years of age (Article I: Table 2). The case fatality rates at seven and 28 days, and at three months were significantly higher among nosocomial cases than among community-acquired cases (for all time frames; p <0.001). The annual rate of mortality within three months increased by 64% during 1995-2001, from 2.6 deaths to 4.2 per 100,000 population per year.

5.2. Combination therapy with levofloxacin in *Staphylococcus aureus* bacteremia (Study II)

5.2.1. Patient characteristics

Patients in the levofloxacin group and in the standard treatment group were well matched with respect to demographic characteristics and predisposing conditions (Article II: Table 1). When the underlying diseases were grouped by the predicted prognoses by McCabe`s classification,[377] 61% of patients had a nonfatal, 27% had an ultimately fatal, and 3% had a rapidly fatal disease. Only 9% of the patients were previously healthy. In both groups the median time from sampling of the first positive blood culture to randomization was three days.

5.2.2. Antibiotic treatment

All patients were treated with an antibiotic that was effective against *S. aureus* from the time of the first positive blood culture. In ITT analysis, parenteral cloxacillin or dicloxacillin was given to 150 of 191 (79%) patients in the levofloxacin group and to 135 of 190 (71%) patients in the standard treatment group (p = 0.09). Only 31 (16%) patients in the levofloxacin group and 42 (22%) patients in the standard treatment group were initially treated with cefuroxime with no significant difference between the groups (p = 0.15). The treatment groups differed neither in the use of clindamycin or vancomycin. Rifampicin was given more frequently to patients in the standard treatment group than in levofloxacin group (77% vs. 65%, respectively; p = 0.01). Combination therapy with an aminoglycoside was also significantly more common in the standard treatment group compared with the levofloxacin group (23% vs. 11%, respectively; p <0.001).

The median duration of parenteral antibiotic therapy from randomization was 29 days (interquartile range [IQR], 22-36 days) in both groups (p = 0.76). Levofloxacin was given for a median of 42 days (IQR, 28-58 days). Total duration of antibiotic therapy, including intravenous and oral dosing, was for a median of 72 days (IQR, 45-85 days) in the levofloxacin group and 80 days (IQR, 42-84 days) in the standard treatment group (p = 0.90).

5.2.3. Clinical manifestations

A skin or soft tissue infection was found in 254 of 381 (67%) patients and no difference was observed between the treatment groups (**Table 5**). At least one deep infection was detected in 331 of 381 (87%) patients during the three months follow-up. Deep infections were definite in 252 (76%) patients and suspected in 79 (24%) patients. Most of these (84%) were diagnosed

within one week after randomization (**Table 5**). A new deep infection after the first week was found equally often in the levofloxacin group and in the standard treatment group (17% vs. 16%, respectively; p = 0.80). The only significant difference in ITT analysis was the lower number of deep-seated abscesses in the levofloxacin group as compared to in the standard treatment group (2% vs. 7%, respectively; p = 0.02). However, this statistical difference was not detected in PP analysis. During follow-up, five (1%) patients had a new SAB more than 28 days after randomization with no significant difference between the groups. Recurrent SAB was due to a relapse in three patients and reinfection in two patients. The infection focus was treated with drainage or surgery in 224 of 381 (59%) patients with no significant difference between the groups.

5.2.4. Outcome

No significant differences in mortality were observed between the treatment groups in ITT or in PP analyses in various subgroups (**Table 6**). The case fatality rate at 28 days was 14% in both study arms and at three months 21% in the standard treatment group and 18% in the levofloxacin group (ITT analysis).

The mean duration of fever (>37.5°C) was nine days in both groups (Article II: Figure 2). A decrease in the rate of serum CRP concentrations were similar in both groups (Article II: Figure 2). No significant differences were observed between the treatment groups in the number of patients with leukocytosis, leukopenia, thrombocytopenia, acidosis, or liver enzyme elevations (data not shown). There were no significant differences in antibiotic-associated diarrhea caused by *Clostridium difficile* or allergic reactions between the treatment groups.

Table 5. Infection foci of 381 patients with *Staphylococcus aureus* bacteremia randomized either to standard treatment or combined with levofloxacin.

Infection focus	From randomization to 1 week					From 1 week to 3 months				
	Levofloxacin group (n = 191)	Standard treatment group (n = 190)	Total (n = 381)	OR (95% CI)	p	Levofloxacin group (n = 191)	Standard treatment group (n = 190)	Total (n = 381)	OR (95% CI)	p
	No. of patients (%)					No. of patients (%)				
Skin or soft tissue	113 (59)	131 (69)	244 (64)	1.53 (1.01-2.34)	0.05	7 (4)	3 (2)	10 (3)	0.42 (0.11-1.66)	0.20
Central intravenous catheter	23 (12)	15 (8)	38 (10)	0.63 (0.32-1.24)	0.18	0 (0)	0 (0)	0 (0)	–	–
Deep-seated abscess	76 (40)	75 (40)	151 (40)	0.99 (0.66-1.49)	0.95	4 (2)	14 (7)	18 (5)	3.72 (1.20-11.51)	0.02
Intramuscular	24 (13)	27 (14)	51 (13)	1.15 (0.64-2.08)	0.64	1 (1)	6 (3)	7 (2)	6.20 (0.74-51.96)	0.06
Epidural or CNS	23 (12)	15 (8)	38 (10)	0.63 (0.32-1.24)	0.18	3 (2)	6 (3)	9 (2)	2.04 (0.50-8.29)	0.31
Other[a]	45 (24)	42 (22)	87 (23)	0.92 (0.57-1.49)	0.74	5 (3)	7 (4)	12 (3)	1.42 (0.44-4.57)	0.55
Pneumonia	65 (34)	66 (35)	131 (34)	1.93 (0.68-1.58)	0.89	12 (6)	9 (5)	21 (6)	0.74 (0.31-1.80)	0.51
Osteomyelitis	54 (28)	66 (32)	116 (30)	1.23 (0.79-1.90)	0.36	9 (5)	5 (3)	14 (4)	0.55 (0.18-1.66)	0.28
Prosthetic device[b]	28 (15)	40 (21)	68 (18)	1.55 (0.91-2.64)	0.10	2 (1)	0 (0)	2 (1)	–	–
Endocarditis[c]	30 (16)	37 (20)	67 (18)	1.30 (0.76-2.21)	0.33	1 (1)	2 (1)	3 (1)	–	–
Septic arthritis	18 (9)	28 (15)	46 (12)	1.66 (0.89-3.12)	0.11	2 (1)	2 (1)	4 (1)	–	–
Urinary tract	12 (6)	16 (8)	28 (7)	1.38 (0.63-2.98)	0.42	0 (0)	0 (0)	0 (0)	–	–
Mediastinitis	10 (5)	11 (6)	21 (6)	1.11 (0.46-2.68)	0.81	0 (0)	0 (0)	0 (0)	–	–
Deep infection[d]	155 (81)	166 (87)	321 (84)	1.61 (0.92-2.82)	0.10	33 (17)	31 (16)	64 (17)	0.93 (0.55-1.60)	0.80

CNS, central nervous system.
[a]Patients with parenchymal, lung, peritoneal, subphrenic, gynecological and pericardial abscesses, or pleural empyema.
[b]Patients with orthopedic, cardiac valve and intravascular devices without peripheral or central intravenous catheters.
[c]Possible or definite endocarditis according to modified Duke criteria.
[d]Patients with deep-seated abscess, pneumonia, osteomyelitis, infection of prosthetic device, endocarditis, septic arthritis, urinary tract infection, mediastinitis, meningitis, septic thrombophlebitis, and recurrent *S. aureus* bacteremia. Each patient has been included once although some patients had several deep infections at various time points.

Table 6. Outcome in various subgroups at 28 days and at three months for 381 patients with *Staphylococcus aureus* bacteremia.

	At 28 days					At 3 months				
	Levofloxacin group	Standard treatment group	Total	OR (95% CI)	p	Levofloxacin group	Standard treatment group	Total	OR (95% CI)	p
Case fatality rate	No./total no. (%)					No./total no. (%)				
Deaths, all	26/191 (14)	27/190 (14)	53/381 (14)	1.05 (0.59-1.88)	0.87	34/191 (18)	39/190 (21)	73/381 (19)	1.19 (0.72-1.99)	0.50
Age										
≤65 years	9/110 (8)	9/113 (8)	18/223 (8)	0.97 (0.37-2.55)	0.95	12/110 (11)	14/113 (12)	26/223 (12)	1.15 (0.51-2.62)	0.73
>65 years	17/81 (21)	18/77 (23)	35/158 (22)	1.15 (0.54-3.67)	0.72	22/81 (27)	25/77 (33)	47/158 (30)	1.29 (0.65-2.55)	0.47
Community-acquired	9/89 (10)	12/85 (14)	21/174 (12)	1.46 (0.58-3.67)	0.42	10/89 (11)	15/85 (18)	25/174 (14)	1.69 (0.71-4.01)	0.23
Hospital-acquired	17/102 (17)	15/105 (14)	32/207 (16)	0.83 (0.39-1.77)	0.64	24/102 (24)	24/105 (23)	48/207 (23)	0.93 (0.50-1.84)	0.91
Diabetes	6/50 (12)	7/53 (13)	13/103 (13)	1.12 (0.35-3.58)	0.85	9/50 (18)	13/53 (25)	22/103 (21)	1.48 (0.57-3.85)	0.42
McCabe´s classification										
Healthy or nonfatal	10/134 (8)	12/134 (9)	22/268 (8)	1.22 (0.51-2.93)	0.66	11/134 (8)	18/134 (13)	29/268 (11)	1.74 (0.79-3.83)	0.17
Ultimately or rapidly fatal	16/57 (28)	15/56 (27)	31/113 (27)	0.94 (0.41-2.14)	0.88	23/57 (40)	21/56 (38)	44/113 (39)	0.89 (0.42-1.89)	0.76
Central intravenous catheter	4/23 (17)	1/15 (7)	5/38 (13)	0.34 (0.03-3.38)	0.34	5/23 (22)	2/15 (13)	7/38 (18)	0.55 (0.09-3.31)	0.51
Endocarditis[a]	8/31 (26)	9/39 (23)	17/70 (24)	0.86 (0.29-2.58)	0.79	10/31 (32)	13/39 (33)	23/70 (33)	1.05 (0.38-2.87)	0.92
Deep infection[b]	24/158 (15)	27/168 (16)	51/326 (16)	0.94 (0.51-1.70)	0.83	31/162 (19)	38/169 (23)	69/331 (21)	0.82 (0.48-1.39)	0.45

[a]Possible or definite endocarditis according to modified Duke criteria.

[b]Patients with deep-seated abscess, pneumonia, osteomyelitis, infection of prosthetic device, endocarditis, septic arthritis, urinary tract infection, mediastinitis, meningitis, septic thrombophlebitis, and recurrent *S. aureus* bacteremia.

In a *post hoc* analysis, the case fatality rate at three months was observed to be significantly higher among those patients with a deep infection who did not receive rifampicin [25 (38%) of 66 patients] than in patients treated with rifampicin [44 (17%) of 265 patients] (OR 3.06; 95% CI 1.69-5.54; p <0.001) (Article II: Table 4). However, patients who did not receive rifampicin were significantly older and significantly more often had hospital-acquired SAB or a severe underlying disease than did those given rifampicin. In contrast, patients not treated with rifampicin had fewer deep infections per patient and fewer cases of endocarditis. Therefore, the effect of rifampicin on mortality was separately analyzed in these patient groups by univariate analysis (**Table 7**). Significantly lower mortality among patients treated with rifampicin as compared to those without it was observed in patients with nosocomial SAB, ultimately or rapidly fatal underlying disease, and those with more than two deep infections per patient (**Table 7**). However, rifampicin had no effect on mortality in patients with age >65 years or endocarditis in univariate analysis. When the effect of rifampicin was adjusted for these subroups (stratified CMH test) rifampicin had a statistically significant lowering effect on mortality in all these subgroups (**Table 7**).

Table 7. Univariate and stratified analyses on mortality at three months in patients with deep infection receiving combination therapy with or without rifampicin.

Variable	Case fatality rate at 3 months			
	Rifampicin (*n* = 265)	No rifampicin (*n* = 66)	Univariate[a] p	Stratified CMH test[b] p
	No./total no. (%)			
Age >65 years	30/99 (30)	15/36 (42)	0.22	0.004
Hospital-acquired	28/128 (22)	17/27 (39)	0.03	<0.001
Ultimately or rapidly fatal disease[c]	23/66 (35)	17/30 (57)	0.04	0.005
More than 2 deep infections per patient	14/113 (13)	8/13 (62)	<0.001	<0.001
Endocarditis[d]	19/62 (31)	4/8 (50)	0.27	<0.001

CMH, Cochran-Mantel-Haenszel.

[a]Chi-square test separately in subgroups for the effect of rifampicin on mortality.

[b]Chi-square test for the effect of rifampicin on mortality adjusted for subgroup variable.

[c]Prognosis or severity of underlying diseases classified according to the criteria of McCabe and Jackson.

[d]Possible or definite endocarditis according to modified Duke criteria.

Furthermore, rifampicin was found to be a confounding factor in the analysis for the effect of levofloxacin on mortality since rifampicin was added significantly more often to patients in the standard treatment group than in the levofloxacin group (p = 0.003) (Article II: Table 4). Therefore, the effect of levofloxacin on mortality in patients who had a deep infection was analyzed separately among those treated with or without rifampicin (stratified CMH test). The case fatality rate at three months among patients with deep infection and rifampicin treatment was 13% (15 of 119 patients) in the levofloxacin group and 20% (29 of 146 patients) in the standard treatment group. Case fatality rates in patients not treated with rifampicin were 37% (16 of 43 patients) and 39% (9 of 23 patients). The benefit of levofloxacin was not statistically significant in this stratified analysis either, in which the imbalance in the use of rifampicin was taken into account (p = 0.16).

5.3. Clinical manifestations and outcome in *Staphylococcus aureus* endocarditis (Study III)

5.3.1. Patient characteristics among injection drug users and nonaddicts

Endocarditis was observed in 74 of 430 (17%) patients with SAB. IE was detected in 20 of 44 (46%) IDUs and in 54 of 386 (14%) nonaddicts (OR 5.12; 95% CI 2.65-9.91; p <0.001). Patients with endocarditis differed from those with SAB only by having significantly more often a predisposing heart disease or a pre-existing liver disease, but less preceding trauma (Article III: Table 1). Other predisposing characteristics and underlying diseases of the patients with IE did not differ from those with SAB.

Among patients with endocarditis, IDUs as a group were younger and had less predisposing heart conditions, coronary artery disease or diabetes than nonaddicts (Article III: Table 2). When the underlying diseases were grouped by the predicted prognoses (McCabe`s classification),[377] none among the IDUs had a rapidly fatal or ultimately fatal disease but they were found in 20 of 54 (37%) nonaddicts (p = 0.001). However, there were three IDUs with HIV infection. Only one drug abuser had a hospital-acquired bacteremia and none associated with the use of central intravenous catheter (Article III: Table 2). Severe sepsis was observed in 9 of 20 (45%) IDUs and in 28 of 54 (52%) nonaddicts without significant difference between the groups.

5.3.2. Antibiotic treatment

Sixty-five of 74 (88%) patients were treated with cloxacillin or dicloxacillin with no significant difference between the groups. Seven (10%) patients received cefuroxime, and one received vancomycin and one ceftriaxone. The median duration of parenteral antibiotic therapy from the first blood culture positive for *S. aureus* was 30 days (IQR, 24-43 days) in IDUs, and 26 days (IQR, 13-34 days) in nonaddicts (p = 0.12), respectively. An aminoglycoside was more often given to addicts than to nonaddicts (95% vs. 54%, respectively; p = 0.001). Eighteen of 20 (90%) IDUs received rifampicin compared with 48 of 54 (89%) nonaddicts with no significant difference between the groups.

5.3.3. Site of endocarditis and echocardiographic findings

According to the modified Duke criteria for endocarditis, 56 (76%) patients were confirmed as definite (10 by pathologic criteria and 46 by clinical criteria), and 18 (24%) patients as possible (**Table 8**). Definite endocarditis was more common among IDUs than in nonaddicts. Left-sided involvement was observed in 93% of nonaddicts whereas in 60% of cases among IDUs right-sided endocarditis (tricuspid valve involvement in all patients) was detected. In nonaddicts, the aortic valve was slightly more often involved (44%) than mitral valve (35%). Prosthetic valve endocarditis, involving the left side only, occurred in 17 of 74 (23%) patients and they all presented in nonaddicts. Most of these patients had an early onset of prosthetic valve IE (12 patients).

Echocardiography was performed in 263 of 430 (61%) patients with SAB. Addicts underwent echocardiography more often (91%) compared with nonaddicts (58%) (OR 7.31; 95% CI 2.56-20.84; p <0.001). In endocarditis, a vegetation was evident by echocardiography in 53% of cases, and a new regurgitation in 71% of cases without significant differences between the groups (**Table 8**). Only four patients had an intracardial abscess or valve perforation.

Table 8. Classification, valvular involvement and echocardiographic findings of injection drug users and nonaddicts with *Staphylococcus aureus* endocarditis.

Variable	Injection drug users (*n* = 20)	Nonaddicts (*n* = 54)	Total (*n* = 74)	OR	95% CI	p
	No. of patients (%)					
Criteria, classification[a]						
Possible	1 (5)	17 (32)	18 (24)	0.12	0.01-0.93	0.03
Definite	19 (95)	37 (69)	56 (76)	8.73	1.08-70.67	0.03
Valvular involvement[b]						
Left-sided	6 (30)	50 (93)	56 (76)	0.03	0.01-0.14	<0.001
Aortic	2 (10)	24 (44)	26 (35)	0.14	0.03-0.66	0.006
Mitral	3 (15)	19 (35)	22 (30)	0.33	0.08-1.25	0.15
Aortic and mitral	1 (5)	7 (13)	8 (11)	0.35	0.04-3.07	0.44
Right-sided	12 (60)	4 (7)	16 (22)	18.75	4.83-72.73	<0.001
Both sides	2 (10)	0 (0)	2 (3)	–	–	0.07
Echocardiography performed	20 (100)	53 (98)	73 (99)	–	–	1.00
TTE	20 (100)	52 (96)	72 (97)	–	–	1.00
TEE	9 (45)	40 (74)	49 (66)	0.29	0.10-0.84	0.03
Echocardiography[c]						
Vegetation	14 (70)	25 (47)	39 (53)	0.26	0.87-7.84	0.16
Regurgitation[d]	17 (85)	35 (66)	52 (71)	2.91	0.75-11.27	0.15
Valve perforation	1 (5)	0 (0)	1 (1)	–	–	0.27
Paravalvular or intracardiac abscess	1 (5)	2 (4)	3 (4)	1.34	0.12-15.67	1.00
Duration of bacteremia before diagnosis, median days (range)	2 (0-8)	3 (0-28)	3 (0-28)	–	–	0.67

TTE, transthoracic echogardiography; TEE, transesophageal echogardiography.

[a]Classified according to modified Duke criteria.

[b]Includes abnormal echocardiographic manifestations both in native valve or in prosthetic valve.

[c]Represents initial echocardiographic findings with TTE alone or with both TTE and TEE.

[d]Regurgitation observed on echocardiogram in any cardiac valve at the time of diagnosis of endocarditis.

5.3.4. Clinical manifestations and outcome

There were no differences in the clinical manifestations between the groups except the tendency for more frequent occurrence of various vascular phenomena among IDUs (Article III: Table 4). The most common infection focus was a skin or a soft tissue infection in 46 of 74 (62%) patients. An extracardiac deep infection was found in 85% of IDUs and in 89% of nonaddicts (p = 0.70). Two patients only developed recurrent bacteremia during the three months follow-up period. Vascular complications, including arterial or venous thromboembolic events, were detected in

60% of IDUs but in only 35% of nonaddicts (p = 0.07) (Article III: Table 4). In particular, septic pulmonary embolism was observed only in IDUs. Whereas all coronary artery related diseases were among nonaddicts, all three parenchymal embolic events were observed in drug abusers. Congestive heart failure in acute phase was present in 11 of 74 (15%) patients with no difference between the groups.

Case fatality rate of all patients with endocarditis was 23% at 28 days, and 31% at three months (**Table 9**). Mortality was significantly higher in nonaddicts than in addicts both at 28 days (OR 8.00; 95% CI 0.99-64.94; p = 0.03), and at three months (OR 5.73; 95% CI 1.20-27.25; p = 0.02). Significant factors for lower mortality in IDUs based on univariate analyses were injection drug abuse (HR 0.22; 95% CI 0.05-0.92; p = 0.04), age (HR 1.03; 95% CI 1.01-1.06; p = 0.006), and none or nonfatal underlying diseases by McCabe`s classification (HR 0.24; 95% CI 0.10-0.54; p = 0.001). Statistically significant association for mortality were not found with the following: right-sided involvement (HR 0.30; 95% CI 0.07-1.30; p = 0.11), left-sided involvement (HR 2.31; 95% CI 0.69-7.78; p = 0.18), severe sepsis at the time of first positive blood culture for *S. aureus* (HR 1.71; 95% CI 0.74-3.95; p = 0.21), or arterial embolic events (HR 2.15; 95% CI 0.94-4.90; p = 0.07). After adjusting by age and underlying diseases, injection drug abuse was not any more significantly associated with lower mortality (HR 0.74; 95% CI 0.10-5.40; p = 0.77).

There were no differences in median duration of fever (three days) or median duration of hospitalization (32 days) between IDUs and nonaddicts (**Table 9**). Cardiac surgery was performed in 15% of IDUs but only in 7% of nonaddicts (OR 2.21; 95% CI 0.45-10.87; p = 0.38). Leukocytosis at the time of first positive blood culture for *S. aureus* was more common in nonaddicts than in IDUs (55% vs. 15%, respectively; p = 0.003), and only two (4%) patients had leukopenia. The median of serum CRP concentration on the day of the first positive blood culture for *S. aureus* was 198 mg/L in addicts compared with 171 mg/L in nonaddicts without a significant difference between the groups. Only two nonaddicts and none of IDUs had ALT level 2-fold above normal limit.

Table 9. Outcome and surgical treatment of injection drug users and nonaddicts with *Staphylococcus aureus* endocarditis.

Outcome	Injection drug users (*n* = 20)	Nonaddicts (*n* = 54)	Total (*n* = 74)	p
	No. of patients (%)			
Case fatality rate				
At 7 days	1 (5)	6 (11)	7 (9)	0.67
At 28 days	1 (5)	16 (30)	17 (23)	0.03
At 3 months	2 (10)	21 (39)	23 (31)	0.02
Duration of fever, median days (IQR)[a]	3 (2-10)	3 (0-6)	3 (1-6)	0.24
Duration of hospitalization, median days (IQR)	32 (30-48)	32 (20-47)	32 (24-47)	0.55
Cardiac surgery	3 (15)	4 (7)	7 (9)	0.38
Valve replacement	1 (5)	4 (7)	5 (7)	1.00
Valve repair[b]	3 (15)	0 (0)	3 (4)	0.02

IQR, interquartile range.

[a]Fever <37.5 $^{\circ}$C after the first positive blood culture for *S. aureus* with 72 patients (two patients excluded, death before defervescence).

[b]Includes two patients with vegetectomy and one patient with annuloplasty.

5.4. Host factors, microbiological and serological characteristics in *Staphylococcus aureus* bacteremia and endocarditis among injection drug users and nonaddicts (Study IV)

5.4.1. Patient characteristics

Into Study IV, all 44 IDUs were included and group matched controls for them as described in the methods section 4.2. Matching was made separately for patients with and without endocarditis. Despite matching, IDUs as a group were significantly younger than nonaddicts (**Table 10**). In addition, IDUs had significantly more frequently community-acquired SAB, a pre-existing liver disease and HIV infection. In contrast, nonaddicts more often had ultimately or rapidly fatal diseases by McCabe`s classification.[377] None of the 88 study patients had previous *S. aureus* deep infection, and prior SAB before the randomization was observed in 14% of IDUs as compared to 2% of nonaddicts (p = 0.11).

Information on drug injection history, drug-use practices, and drugs were available from 29 IDUs. A high proportion of IDUs (22 of 29 patients [76%]) injected amphetamine, although most of them (97%) used many drugs. Heroin was injected by 13 of 29 addicts (45%), but only two patients used cocaine. Ten of 29 IDUs (34%) reported sharing of paraphernalia (e.g., needles, spoons, and filters) for the drug use.

Table 10. Characteristics of injection drug users (cases) and nonaddicts (controls) with *Staphylococcus aureus* bacteremia.

Characteristic	Injection drug users (*n* = 44)	Nonaddicts (*n* = 44)	Total (*n* = 88)	OR	95% CI	p
No. of patients (%)						
Age (years, mean ± SD)	29 ± 8	50 ± 19	40 ± 18	–	–	<0.001
Male gender	32 (73)	28 (64)	60 (68)	1.52	0.62-3.76	0.49
Hospital-acquired	3 (7)	25 (57)	23 (32)	0.06	0.02-0.21	<0.001
Predisposing factor						
Previous skin disease or wound	26 (59)	25 (57)	51 (58)	1.09	0.47-2.56	1.00
Predisposing heart condition	4 (9)	11 (25)	15 (17)	0.30	0.09-1.03	0.09
Congenital heart disease	3 (7)	2 (5)	5 (6)	1.54	0.24-9.68	1.00
Prior endocarditis	1 (2)	3 (7)	4 (5)	0.32	0.03-3.18	0.62
Degenerative heart disease	0 (0)	6 (14)	6 (7)	–	–	0.03
Prosthetic valve	0 (0)	6 (14)	6 (7)	–	–	0.03
Alcoholism	2 (5)	2 (5)	4 (5)	1.00	0.14-7.43	1.00
Prior colonization of *S. aureus*[a]	1 (2)	8 (18)	9 (10)	0.11	0.01-0.88	0.03
Prior surgery in 3 months	0 (0)	10 (23)	10 (11)	–	–	0.001
Central intravenous catheter	0 (0)	8 (18)	8 (9)	–	–	0.006
Corticosteroid use ≥1 month	0 (0)	4 (9)	4 (5)	–	–	0.12
Immunosuppressive therapy[b]	0 (0)	2 (5)	2 (2)	–	–	0.49
Underlying disease						
Liver disease	36 (82)	5 (11)	41 (47)	35.10	10.51-117.2	<0.001
HIV positive	7 (16)	0 (0)	7 (8)	–	–	0.01
Diabetes	3 (7)	12 (27)	15 (17)	0.20	0.05-0.75	0.02
Coronary artery disease	0 (0)	8 (18)	8 (9)	–	–	0.006
Chronic renal failure	0 (0)	7 (16)	7 (8)	–	–	0.01
Malignancy	0 (0)	4 (9)	4 (5)	–	–	0.12
McCabe`s classification[c]						
Healthy or nonfatal disease	44 (100)	33 (75)	77 (88)	1.00	Ref.	–
Ultimately or rapidly fatal disease	0 (0)	11 (25)	11 (13)	–	–	<0.001

[a]Includes only skin colonization before randomization.

[b]During six months preceding the positive blood culture.

[c]Prognosis or severity of underlying diseases classified according to the criteria of McCabe and Jackson.

5.4.2. Clinical manifestations and outcome

There were no differences in the clinical manifestations between IDUs and nonaddicts (n = 88) except the more frequent infections due to a central intravenous catheter and a prosthetic device among nonaddicts (Article IV: Table 2). The most common infection focus was a skin or a soft tissue infection observed in 61 of 88 (61%) patients with no significant difference between the patient groups. There was a trend, although it did not reach statistical significance, that a deep infection was found more often among IDUs (98%) than in nonaddicts (86%) (p = 0.06). No differences were observed in the occurrence of thromboembolic events, duration of fever, serum CRP concentration at the time of the first positive blood culture for *S. aureus* (data not shown), or proportion of patients with leukocytosis (data not shown) between IDUs and nonaddicts. However, addicts had significantly shorter median duration of hospitalization (31 days) compared with nonaddicts (43 days). There were six deaths (two IDUs and four nonaddicts) during three months follow-up, all with endocarditis.

In endocarditis (n = 40), left-sided involvement was significantly more frequent in nonaddicts (90%) whereas right-sided endocarditis predominated among addicts (60%) (Article IV: Table 2). Septic pulmonary embolism was observed only in addicts.

5.4.3. Bacterial strains

Among the 87 *S. aureus* isolates available (one bacterial strain isolated from an addict could not be obtained for further analysis), a total of 24 different strain types were detected by PFGE (**Table 11**). Seven of the genotypes were similar to known epidemic MRSA types (indicated with FIN-names), and eight were similar to known MSSA types. Nine strains possessed a sporadic PFGE profile. Of the 19 strains isolated from IDUs with endocarditis, 15 (79%) showed a PFGE profile of a known MRSA genotype. Similarly, among the 24 strains from IDUs without endocarditis, 17 (71%) strains shared a PFGE profile of a known MRSA genotype. Among the 20 strains from nonaddicts with endocarditis, 11 different PFGE profiles were found (nine MRSA or MSSA strain types and two sporadic profiles). Fourteen (70%) strains possessed a known MRSA PFGE profile. The 24 strains isolated from nonaddicts without endocarditis distributed to 11 profiles.

Bacteremia caused by either a FIN-4 or a FIN-14 strain was more often detected among IDUs (42%) than among nonaddicts (18%) (OR 3.24; 95% CI 1.22-8.60; p = 0.02). FIN-4 strain was the only single strain observed more frequently in IDUs (21%) than in nonaddicts (5%) (OR 5.56; 95% CI 1.13-27.46; p = 0.03). No single strain was statistically associated with endocarditis.

S. aureus strains (*n* = 87) isolated from IDUs and nonaddicts were equally often SAK, haemolysin and protease producers (Article IV: Table 4). Similarly, there were no differences either in the proportion of SAK production, haemolysis and protease activity in endocarditis between IDUs and nonaddicts. However, there was a slight trend that haemolytic properties were found more often from SAB patients without IE than with IE (79% vs. 59%, respectively; p = 0.06). This difference was specifically clear among IDUs without endocarditis than with endocarditis (88% vs. 47%, respectively; p = 0.007). In contrast, no differences were detected in protease production in the above patient groups. The PVL-gene was detected in only two of 87 (2%) strains. Both of these were of MSSA8 PFGE profile; one was observed in an addict without endocarditis, and the other in a nonaddict with endocarditis.

Table 11. Genotypes of *Staphylococcus aureus* isolates (*n* = 87) among injection drug users (cases) and nonaddicts (controls) with and without endocarditis.

	Injection drug users		Nonaddicts	
PFGE	Endocarditis (*n* = 19)	Without endocarditis (*n* = 24)	Endocarditis (*n* = 20)	Without endocarditis (*n* = 24)
	No. of patients (%)		No. of patients (%)	
FIN-10	2 (11)	4 (17)	4 (20)	9 (38)
FIN-14	4 (21)	5 (21)	4 (20)	2 (8)
FIN-4[a]	4 (21)	5 (21)	–	2 (8)
FIN-5	5 (26)	2 (8)	1 (5)	1 (4)
MSSA5	–	2 (8)	2 (10)	3 (13)
FIN-16	–	–	2 (10)	2 (8)
FIN-7	–	1 (4)	2 (10)	–
Other types[b]	4 (21)	5 (21)	5 (25)	5 (21)

[a]Significantly more frequent in the 43 injection drug users compared with the 44 nonaddicts (p = 0.03).
[b]Includes PFGE types FIN-3, MSSA1, MSSA3, MSSA8 (PVL+), MSSA10, MSSA11, MSSA14 and MSSA22 (1-2 strains each) and sporadic MSSA types (*n* = 9).

5.4.4. Serological assays

Serum specimens from 85 patients at acute phase and from 80 patients at convalescent phase could be obtained for further analyses. Serum samples from three addicts at acute phase and from eight addicts at convalescent phase could not be obtained due to compliance problems. A positive ASTA titer was detected at acute phase in 17 of 85 (20%) SAB patients and at

convalescent phase in 33 of 80 (41%) patients. Similarly, an initially positive titer for TAA was found in 9 of 85 (11%) patients and at convalescent phase in 17 of 80 (21%) patients. The combined use of these tests (positive TAA or ASTA at acute or convalescent phase, or 4-fold or greater rise in them) increased seropositivity in all SAB patients and in endocarditis (59% vs. 62%, respectively).

The serological responses of TAA differed between IDUs and nonaddicts (**Table 12**). TAA titers were significantly more often elevated in IDUs than in nonaddicts both at acute phase (OR 10.42; 95% CI 1.24-87.52; p = 0.01) and at convalescent phase (OR 5.65; 95% CI 1.65-19.39; p = 0.005). This difference was found especially in endocarditis (**Table 12**). Interestingly, a positive ASTA titer at acute phase was found significantly more frequently among IDUs without IE than with IE (44% vs. 6%, respectively; p = 0.01).

Table 12. Positive serological tests among injection drug users (cases) and nonaddicts (controls) in *Staphylococcus aureus* bacteremia, especially in endocarditis.

Serological test	All cases and controls		Endocarditis	
	Injection drug users	Nonaddicts	Injection drug users	Nonaddicts
	No./Total no. (%)		No./Total no. (%)	
ASTA ≥3.2 U/ml				
Acute phase	11/41 (27)	6/44 (14)	1/18 (6)	2/20 (10)
Convalescent phase	15/36 (42)	18/44 (41)	5/18 (28)	9/20 (45)
ASTA 4-fold rise	7/35 (20)	15/44 (34)	3/17 (18)	9/20 (45)
TAA ≥8				
Acute phase	8/41 (20)[a]	1/44 (2)	6/18 (33)[c]	1/20 (5)
Convalescent phase	13/36 (36)[b]	4/44 (9)	9/18 (50)[d]	2/20 (10)
TAA 4-fold rise	4/35 (11)	4/44 (9)	2/17 (12)	1/20 (5)

ASTA, antistaphylolysin; TAA, teichoic acid antibody; IDUs, injection drug users.

[a]Significantly more frequent in IDUs compared with nonaddicts (p = 0.01).

[b]Significantly more frequent in IDUs compared with nonaddicts (p = 0.005).

[c]Significantly more frequent in endocarditis among IDUs compared with nonaddicts (p = 0.04).

[d]Significantly more frequent in endocarditis among IDUs compared with nonaddicts (p = 0.01).

6. DISCUSSION

6.1. Epidemiology of *Staphylococcus aureus* bacteremia

S. aureus is the second most common bloodstream isolate both in hospital and community-acquired bacteremias in all age groups.[42,56,77,382] The epidemiology of SAB has mostly been studied in selected hospitals, which may not be representative for the whole population.[7,17,44-46] In addition, only a few population-based studies, which identify trends in the incidence and outcome over time and regions, have previously been published.[51] The age- and sex-specific incidence rates have rarely been reported.[48,50-52] Most recent studies have focused on the contribution of methicillin resistance to morbidity and mortality associated with SAB.[15,49,53,54] However, the prevalence of methicillin resistance among *S. aureus* blood isolates varies widely between countries, regions, hospitals and different wards of the same hospital.[232,383,384] Population-based studies allow comparisons between countries and will provide further insight into the changes in the epidemiology of SAB. This might help assessing the impact of the ongoing MRSA epidemic occurring worldwide, and give more information on the relative importance of nosocomial and community-acquired SAB.[51]

In Finland, *S. aureus* bloodstream infections are mostly caused by methicillin-sensitive strains and the prevalence of bacteremic MRSA infections has remained low (<3%).[90,91] Our nationwide population-based study (Study I) demonstrated that the annual incidence of SAB rose significantly by 55%, from 11 to 17 cases per 100,000 population during 1995-2001. This incidence is still at a lower level than rates previously reported from Denmark and the United States,[10,48,50,52] but similar to those in the UK, Ireland, and Canada.[49,51,53,54] A major increase in SAB rates observed in Denmark during 1957-1991, a time period that preceded all the other population-based studies, occurred in hospitals while the community-acquired cases remained at almost the same level.[48,50] In the 1990s, the overall SAB incidence in European countries and Canada were of a similar magnitude as in our study, but it was double in a metropolitan area in the United States.[48-54] Previously, MRSA has been concluded to contribute significantly to the increase in the overall incidence of SAB.[44,49] We observed a major increase during a relatively short time period in the absence of invasive MRSA. In all other population-based studies, except the one from Canada,[51] the proportion of MRSA out of bacteremic *S. aureus* infections was considerable, ranging from 15% to 43% in the 1990s.[48-54]

In addition, rates of SAB may vary because of differences in demographics of the at-risk population, blood culture ordering practices, laboratory methods, and use of empirical antibiotic therapies. In our study as well as in the previous longitudinal studies from Denmark and the UK, the major increase in incidence occurred in the older age group.[48-50] In studies with shorter observation periods, the highest incidences have been found among males and persons ≥65 years old,[51,52] which were also the biggest patient groups in our nationwide prospective Study II. Except for children <1 year old, the risk steadily increased with age in all studies.[48-52]

Hospital-based studies suggest that rates of both community- and hospital-acquired SAB have been increasing[44,77,78] although SAB has been predominantly a nosocomial infection over the decades.[50,52,79-81] In Study I, the proportion of hospital-acquired SAB predominated but remained stable over time (**Table 3**), and was unexpectedly similar to that presented in Study II (54%) and in other previous studies (46-53%).[8,51,52,385] In our definition of nosocomial origin (Study I), we presented SAB in recently discharged patients and in patients admitted from other institutions, such as nursing homes, from being erroneously classified as community-acquired. Data on SAB associated with invasive procedures performed just before or at the time of admission were not available.

Several factors have been suggested for the increase the risk of invasive *S. aureus* infections. A high proportion of patients with SAB generally have one or more underlying diseases.[1,51,84,119-121] Study I did not include data on risk factors or comorbid conditions of patients with SAB. However, our nationwide Study II showed that most common underlying diseases were cardiovascular diseases (47%), diabetes (27%), chronic lung diseases (19%), chronic renal failure (16%), and hepatic cirrhosis (15%) in accordance with earlier reports.[1,51,84,119-121]

In Study I, we observed temporal and geographic differences in the population-based blood culture sampling rates. The rate of blood culture sampling rose by 21%, from 28 blood-culture sets to 35 per 1000 population during 1995-2001. Previously, the Danish study reported a 4-fold increase in the use of blood cultures from 1957 to 1991.[50] In South and West England in 1995, blood culture sampling rates varied 2.5-fold between geographic areas, and variations in the incidences of invasive pneumococcal disease were largely related to variations in blood culture sampling rates.[386] The increased incidence of SAB in Study I might be at least due to increased reporting. However, it is unlikely that improved reporting accounted for the entire increase, as there was an increased incidence in adult age groups in the absence of an increase among

children. Furthermore, the increasing trend could be related to increased utilization of blood cultures, attributed to a more seriously ill patient population. We did not have data on blood cultures processed by age and sex, and therefore we could not assess the relationship between blood culture sampling rate and age.

In Study I, case fatality rates were reported at seven days and 28 days, and at three months from the first positive blood culture specimen for *S. aureus* by using data from the National Population Registry, but we did not consider the status at discharge (in-house mortality), which might be affected by the length of stay and healthcare delivery policies. Overall, 17% of SAB cases died within 28 days which is less than the mortality of 23% to 39% generally related to SAB today.[1,3,7] However, a recent nationwide survey from Denmark,[10] a country with a very low prevalence of MRSA, suggests that mortality in SAB has decreased during 30 years, which might be one explanation for the lower case fatality rate in Study I when compared with earlier data. Furthermore, a direct comparison of SAB mortality with various reports is complicated by inconsistent definitions and variable analysis time points. The relatively low mortality rates have been based on the definition of death within seven days after the onset of SAB (i.e., mortality directly attributable to SAB).[12,45,221,222] In other studies, higher mortality rates have been reported due to longer time period of death within two to five weeks after the onset of bacteremia (i.e., in-hospital or overall mortality).[3,7,8,120,135] Alterations in case fatality rates suggest the need to standardize the mortality end-points.

In Study I, the mortality was extremely low among children and young adults, but it increased with age, being 37% at 28 days among persons aged >74 years. The case fatality rate at 28 days for nosocomial cases was almost double than that for community-acquired cases (22% vs. 13%), and did not change over time. This could be due to a higher age and probably a more frequent prevalence of severe underlying diseases in nosocomial cases. These results are comparable with Study II, where the mortality at 28 days increased among older patients (22%) and was higher in nosocomial cases than in community-acquired cases (16% vs. 12%).

The study from Canada also documented the annual mortality rate due to invasive *S. aureus* infections (5/100,000 population per year during 1999-2000).[51] The rate is higher than ours (4/100,000 per year in 2001), likely due to the fact that their study included all sterile sites and we only had bacteremias. We observed an annual increasing trend in SAB mortality rate (2.6 to 4.2 deaths per 100,000 population during 1995-2001), which has also been reported from

England and Wales during 1993-2002.[49] However, the UK study was based on death certificates, and MRSA accounted for most of the increase detected.

The data in the epidemiologic Study I offered a comprehensive assessment of trends and outcome of SAB in a well-defined population during seven years period, although we did not have data on risk factors for acquisition of SAB, underlying conditions, and other complications besides death. Interestingly, the changes we detected in the epidemiology of SAB are very similar to those found in countries with a high prevalence of MRSA. The increased incidence in Finland might be due to a growing population at risk, affected by such factors as high age and the prevalence of chronic diseases, and improved survival of patients with severe comorbidity in both hospital and non-hospital settings.

6.2. Effect of antibiotic treatment on outcome in *Staphylococcus aureus* bacteremia

6.2.1. Mortality

S. aureus bacteremia still today confers high mortality and frequent morbidity, although antistaphylococcal antibiotics have been available for more than 40 years.[3,6,9-14] The possibility of MRSA will not have to be considered in Finland when initiating antibiotic treatment. Therefore, the standard treatment strategy for bacteremic *S. aureus* infections is based on a semisynthetic penicillin. Data on the effect and recommendations of various antibiotic combinations are controversial, although they are widely used in SAB with deep infections. An aminoglycoside is combined with standard treatment in endocarditis for 3-14 days, and addition of rifampicin is variably used in deep infections or because of poor response to the standard treatment.[28,33-35,269] Some small clinical studies have shown that combination therapy with rifampicin improves clinical cure and bacteriological eradication whereas no effect in mortality has been seen.[30,31,268,269]

The new treatment options such as newer fluoroquinolones with improved activity against gram-positive organisms could be available for severe staphylococcal infections. In experimental studies, fluoroquinolone combined with standard therapy has shown improvement in treatment results.[38] Because most deep infections are observed within two weeks after the onset of bacteremia,[39] it was speculated that especially these metastatic infections might be prevented by early treatment with bactericidal fluoroquinolone due to its excellent penetration into tissues. Therefore, we conducted the prospective clinical trial (Study II) where efficacy of levofloxacin in

addition to standard treatment of SAB was studied in relation to patient outcome and development of deep infections. Levofloxacin was chosen since it was the only available fluoroquinolone with enhanced gram-positive activity given both orally and intravenously at that time in Finland.

Levofloxacin combination therapy did not decrease mortality nor did it speed up recovery (**Table 6**). Overall, 14% of SAB patients died within 28 days in Study II. The low mortality is comparable with that observed in recent population-based studies from Finland (Study I) and Denmark,[10] but clearly higher mortality rates have been reported as discussed above (see section 6.1.).[1,3,7] In addition, all patients in Study II were followed by an infectious disease specialist, which has been shown to improve the outcome and reduce the number of relapses.[7,11] Mortality at 28 days in SAB (Study II) was highest among patients with ultimately or rapidly fatal disease (27%), endocarditis (24%), over 65 years old (22%), deep infection (16%), and hospital-acquired bacteremia (16%) in agreement with earlier reports.[3,7,14,16,121,145] Interestingly, mortality of patients with a deep infection was only 16% although higher mortality rate of 28% has been observed previously.[16] No differences in mortality of these various subgroups could be observed between the antibiotic treatment groups (**Table 6**).

In Study II, rifampicin was included in the protocol for all patients with deep infection since the ultimate aim was to evaluate if levofloxacin improved treatment results when the best therapy was used. Interestingly, if rifampicin was not given mortality was significantly higher than among those who received rifampicin (38% vs. 17%). However, this result must be interpreted with caution, because the trial was not specifically designed to scrutinize the effect of rifampicin. Older age, nosocomial bacteremia, and ultimately or rapidly fatal disease by McCabe`s classification were associated with higher mortality in patients not receiving rifampicin which has been confirmed generally in previous reports.[7,14,65,145] Furthermore, endocarditis and high frequency of deep infections were related to poor prognosis.[5,7,10,13,16,134] Although these variables occurred more often among patients treated with rifampicin, significantly higher mortality was still observed among those not treated with rifampicin. In conclusion, patients with a deep infection appeared to benefit from combination treatment with rifampicin, as suggested earlier only by experimental data and small clinical studies.[30,31,268,269] Thus, we could assume that addition of rifampicin in patients with a deep infection might improve outcome, especially in patient groups which are generally associated with poor prognosis.

In the levofloxacin group there were significantly more patients with a deep infection not treated with rifampicin (27%) as compared to the standard treatment group (14%). The reasons for not using rifampicin were concomitant liver disease in 15 cases (nine patients in the levofloxacin group vs. six patients in standard treatment group), risk of a drug interaction or other decision of the treating physician in 47 cases (31 vs. 16 patients), and an early death of the patients in four cases (three vs. one patients). The stratified CMH test was used in order to adjust for levofloxacin as a confounding factor when the effect of rifampicin was analyzed. In patients with deep infection receiving rifampicin, a trend towards lower mortality (13%) was observed in the levofloxacin group as compared to the standard treatment group (20%). This difference, however, was not statistically significant. The data indicate that a fluoroquinolone could not be recommended to be combined with the standard treatment of SAB. As the present study design did not directly compare levofloxacin to rifampicin, the potential benefit of levofloxacin when rifampicin cannot be used remains to be shown in further prospective studies. If fluoroquinolones could be useful in MRSA bacteremias cannot be answered by this trial, because bacteremias due to MRSA were not included. Resistance to fluoroquinolones has increased among MRSA strains,[95] why they, however, might not offer a good therapeutic alternative for the treatment of MRSA infections.[292]

6.2.2. Clinical manifestations

Study II provides many unique features: the prospective design for three years in 12 Finnish hospitals, worldwide one of the largest population of SAB patients, and comprehensive data of antibiotic treatment, clinical manifestations and outcome of SAB in area with a very low prevalence of methicillin resistance. The initial infection focus for SAB is most often skin or soft tissue infection which was observed in two thirds of all patients during the three months follow-up in Study II. However, the clinical impact of SAB is determined by its complications, particularly by the development of deep infections due to metastatic spread, and by high recurrence rate of bacteremia.[1,3-7] The reported frequency of metastatic infections varies widely, from 10% to 53%,[9,16,84,121,145] but it was even 87% in Study II. This difference might be partly explained by different definitions between studies as well as by the high intensity search for deep infections in our study. Furthermore, in most articles the incidence of deep infections have not been separately reported or they have been classified into primary and secondary foci (i.e., metastatic infections).[7,17,121,123,128,138]

In Study II, infection foci were analyzed according to whether observed before or later than one week after randomization predicted the effectiveness of levofloxacin therapy as compared to the standard treatment (**Table 5**). Most of the deep infections (84%) were diagnosed within one week after randomization. In another recent study,[16] 74% of metastatic infections were already present at the time of hospitalization in accordance with our findings. Thus, these data suggest that infection foci cannot reliably be classified as primary or metastatic. In addition, there was no significant difference in the formation of new deep infections after the first week of randomization between the treatment groups. This might be one reason why levofloxacin combination therapy did not decrease the incidence of deep infections which was one of the main objectives for the study.

Intravenous antibiotic treatment is recommended for four to six weeks in SAB with a deep infection.[76,86] In Study II, the duration of parenteral and oral antibiotic therapy was much more prolonged due to high incidence of deep infections, and extended with an average of 77 days. Of all patients, 44% remained on antibiotic treatment at three months. This may have contributed to the low (1%) prevalence of SAB recurrences (three patients with relapse and two with reinfection). Significantly higher recurrence rates from 9% to 23%, have been reported in studies with slightly longer follow-up times from three to six months.[6,8,11,13,16,65,235]

Our study suggests that when SAB occurs, identification of deep infections might be essential for decreased mortality, and therapeutic approach including focus eradication and prolonged parenteral therapy with deep infections are warranted.

6.3. Endocarditis

6.3.1. Predisposing factors and underlying conditions for endocarditis

S. aureus is a leading cause of bacteremia and infective endocarditis (IE) in many regions of the world.[18,21,76,126] During recent decades IE has been observed in 11% to 35% of SAB attributable to different sets of diagnostic criteria.[65-68] In agreement with previous data, the incidence of endocarditis was 17% of all SAB patients in Study III. Changes in the epidemiology of IE have emerged. Classic risk factors such as rheumatic heart disease are being replaced by new ones, including IDUs, elderly patients with degenerative valve disease, patients with intravascular catheter or prosthetic valve, mitral valve prolapse, prior IE, and nosocomial acquisition.[22,79,126] This was also confirmed in Study III, despite that *S. aureus* endocarditis was primarily community-acquired in 54% of patients as reported earlier.[84,123]

S. aureus endocarditis presents as two distinct patient groups, IDUs and nonaddicts, depending on the different location of infection, and differences in clinical manifestations and prognosis.[120] The incidence of endocarditis in SAB varies between 35% to 67% among IDUs.[65,69-72] IE was found in 46% of IDUs in our study (Study III). Among patients with SAB and endocarditis (Studies III and IV), IDUs as a group were significantly younger, had less predisposing heart conditions or severe underlying diseases by McCabe`s classification,[377] and had more often community-acquired bacteremia as published previously.[19,71,145,157,179,208,358,387] Furthermore, IDUs had significantly more often pre-existing liver disease (mainly chronic hepatitis C infection) than nonaddicts, but despite that none of the addicts had an abnormal ALT elevation during the three months follow-up.

The reasons for the high occurrence of *S. aureus* endocarditis among IDUs are largely unknown. Factors that seem to contribute to an increased prevalence of staphylococcal disease in addicts include the pathogen, the host, the drugs (e.g., heroin or cocaine), the drug-use environment, and drug using habits.[126] The source of *S. aureus* may be endogenous (the drug user´s own flora) or external (contaminated drugs, drug adulterants, or paraphernalia).[182,183,208,388] However, previous *S. aureus* deep infection or bacteremia before the randomization was not associated with the present SAB or endocarditis among IDUs (Studies III and IV). Prior *S. aureus* colonization of the skin was significantly more often observed in nonaddicts than among IDUs (Study IV) which is in contrast with previous data.[111,112]

6.3.2. Site of endocarditis and clinical manifestations

S. aureus endocarditis is associated with a higher occurrence of extracardiac deep infections due to metastatic spread and thromboembolic events compared with IE caused by other pathogens.[18,21] The differences between IDUs and the general population have been derived from non-comparative trials, and there are only a few studies where the clinical picture of *S. aureus* endocarditis in these patient groups has been compared.[19,21,189,224] Therefore, we collected prospectively one of the largest SAB population with endocarditis comparing patients with and without injection drug abuse (Studies III and IV). The lack of MRSA might complicate extrapolation of these results into countries with a high MRSA prevalence. However, MRSA endocarditis is usually more difficult to treat. While concentrating only on cases by MSSA strains real differences between IDUs and nonaddicts might be better revealed.

The proportion of definite IE (76%) in Study III (**Table 8**) was in line with that observed in the other large survey.[65] Frequent use of echocardiography may increase the incidence of endocarditis at least to some extent. In our study, echocardiography was performed as clinically indicated. It was done to 61% of SAB patients (Study III) and the rate was similar or higher compared with other studies.[7,16,189] Some endocarditis cases with an atypical presentation might have been missed because echocardiography in our cohort as well as in previous studies was not performed for all SAB patients.

In Study III, the tricuspid valve was predominantly affected in addicts (60%), although the incidence was slightly lower than that reported previously (from 70% to 90%) (**Table 8**).[19,79,189,193] Furthermore, the frequency of left-sided involvement in addicts was as high as 30% which is similar to that observed in other studies,[64,79,196] but higher than in most earlier reports with left-sided endocarditis ranging from 8% to 19%.[71,186,193] In IDUs, both sides of the heart are usually involved simultaneously in 5% to 10% of cases as detected also in our trial.

The increased risk of soft tissue abscesses has been related to the use of a cocaine and heroin mixture.[63,111,388] In Study IV, most IDUs had injected amphetamine and only two patients had used cocaine. This suggests that the risk for soft tissue infections might not be associated with the abused drug but rather to the injection mode of use. Extracardiac deep infections are generally observed in left-sided endocarditis with an incidence of 40% to 76%.[12,69,164] In contrast to previous studies,[19,71,145] extracardiac deep infections occurred in over 80% in both IDUs and nonaddicts (Studies III and IV). Systemic thromboembolic events are generally found in 21% to 50% of patients in endocarditis[168-170,172] and they are related especially to left-sided involvement and prosthetic heart valves with high mortality rates. In earlier reports, IDUs had fewer arterial emboli or strokes than nonaddicts probably due to involvement of right-sided endocarditis.[21,185,197] In contrast, we observed arterial thromboembolic events equally often among addicts and nonaddicts (Studies III and IV). Venous embolic events were even more common in IDUs, and septic pulmonary embolism manifestated only in IDUs. However, the frequency of septic pulmonary embolism (40%) was less than the previously reported incidence of 67% to 87% among addicts.[185,194,195] Recurrent endocarditis is frequently seen in IDUs, and the median intervals between episodes is far shorter in addicts compared with nonaddicts.[170]

The duration of antibiotic treatment in SAB depends largely on the presence of an associated IE, in which four to six weeks therapy is recommended.[27,86,157] A shorter two weeks treatment

has been suggested for selected cases of right-sided involvement with a good prognosis.[27,389] However, the high proportion of extracardiac deep infections among addicts extended the intravenous antibiotic therapy to four weeks (Study III). This observation suggests that deep infections should be actively searched for also in IDUs.

6.3.3. Mortality in endocarditis

The overall mortality in *S. aureus* endocarditis is high, ranging between 30% to 71% according to various data.[18,60,65,66,124,143,224] In Study III, a lower mortality (23%) at 28 days was found in endocarditis due to SAB (**Table 9**). Mortality at three months was also only 31%. The prognosis of staphylococcal endocarditis among IDUs tends to be better than in nonaddicts, with mortality rate varying between 2% and 12%.[19-21] This was also confirmed in our study (Study III), where the mortality for IDUs with endocarditis was only 5% whereas 30% of nonaddicts deceased (**Table 9**).

Fatal outcome in *S. aureus* endocarditis has been associated with older age, rapidly fatal underlying diseases, pre-existing heart valve diseases, presence of a prosthetic valve, severe sepsis, left-sided involvement, CNS events, heart failure, absence of surgical therapy, and persistent bacteremia.[7,18,21,65,68,189,224,225] In contrast, host factors such as younger age and lack of severe underlying diseases in addicts have been associated with favourable prognosis,[19,21,157,224] which was also observed in Study III. Furthermore, the reason for better outcome in addicts has been partly explained by observations in experimental right-sided endocarditis.[226,227,390,391] Spontaneous sterilization of valve vegetations were detected more often in the right side of heart and the density of bacteria in infected tricuspid vegetations was smaller than in left-sided valves. Study III showed the tendency for lower mortality in patients with right-sided IE but for higher mortality in patients with severe sepsis, left-sided involvement, and arterial embolic events. However, these factors did not achieve statistical significance probably due to insufficient sample size.

There is no single hypothesis to explain why addicts have better outcome than nonaddicts in *S. aureus* endocarditis. The findings of an equal number of extracardiac deep infections and vascular phenomena among IDUs (Studies III and IV) makes it even more difficult to find an explanation. However, we showed that injection drug abuse in accordance with younger age and lack of severe underlying diseases were associated with better prognosis as published previously. In addition, a favourable outcome in Study III could have been due to an intensive search for extracardiac deep infections which led to prolonged intravenous antibiotic therapy also in addicts.

6.3.4. Bacterial strain characteristics and serological assays

Outbreaks due to a single *S. aureus* strain have been reported in addicts.[183,349] In Study IV, FIN-4 strain was the only single strain observed significantly more frequently among IDUs as compared to nonaddicts, but no epidemic clone was detected (**Table 11**). The other strain that slightly dominated among IDUs was FIN-14 and when analyzed together with FIN-4 they were found more often among IDUs than among nonaddicts. The similar strains have previously been related to community-acquired MRSA infections.[353] However, bacteremia and endocarditis among IDUs were caused by many various strains and these strains were commonly found among nonaddicts as well (Study IV). Therefore, no individual strain dominated as an etiologic agent for endocarditis in either patient groups.

It has been speculated that frequent encounter with a microbe, or bacterial strain characteristics play a role in the development of endocarditis, and also possibly a more prevalent serologic response among IDUs. Staphylokinase (SAK) production is connected to an uncomplicated bacteremia and a better prognosis.[333] SAK has been suggested to be part of the adaptive mechanisms of *S. aureus* favoring bacterial symbiosis with the host or preventing defence mechanisms of the host. These hypotheses were not supported by the present Study IV, because SAK expression could be demonstrated in 87% of all strains, and even in 92% of strains associated with deep infections (i.e., complicated SAB). There were neither any significant differences in the ratios of protease production among strains originating from various patient groups. Interestingly, although there was no differences in haemolysin production between strains from IDUs and nonaddicts, haemolytic properties were found significantly more frequently in strains from IDUs without endocarditis than with endocarditis (88% vs. 47%). It would be possible that *S. aureus* strains without haemolytic activity might predispose to endocarditis in addicts. This finding warrants further studies.

S. aureus strains producing Panton-Valentine leukocidin (PVL) are related to community-acquired skin infections and severe pulmonary manifestations in children and young adults.[209,339,392] We observed in Study IV, that majority of isolates (98%) were PVL-negative although two thirds of the IDUs and nonaddicts had a skin or a soft tissue infection. Our results are in accordance with a previous report, in which PLV was neither associated with deep infections such as endocarditis.[209]

Classical serological markers for *S. aureus* infections are antibody levels against α-haemolysin (ASTA) and ribitol teichoic acid (TAA). In earlier reports, the elevated TAA values were seen in SAB with metastatic infections such as endocarditis.[69,250,357,366] ASTA is considered to be of limited value due to its low sensitivity. High titers of ASTA have been found in staphylococcal patients with various dermatoses, particularly in atopic dermatitis.[373,374] According to Study IV, TAA and ASTA were not helpful in identifying deep infections in SAB because only a small proportion of patients developed a positive serological response. However, positive TAA titers during the acute phase of bacteremia were found among addicts, especially in IE. Furthermore, half of the IDUs with endocarditis had a positive TAA titer also at the convalescent phase. Thus, this serological response most probably was due to previous and recurrent intravenous exposure to *S. aureus* in agreement with earlier reports.[362,368]

Interestingly, we detected an elevated initial ASTA titer significantly more often among IDUs without endocarditis than with endocarditis (44% vs. 6%). The same patient group also displayed a higher percentage of elevated ASTA titers during the course of the disease. These findings support the idea that addicts frequently "vaccinate" themselves with *S. aureus* creating an antibody response against α-haemolysin which in turn might protect from endocarditis and give better prognosis in SAB.

7. SUMMARY AND CONCLUSIONS

The main results of the present study can be summarized:

I   The annual incidence of SAB in Finland during 1995-2001 increased in our retrospective epidemiologic study. Increased incidence was especially observed in elderly people. While the increase in incidence may partly be explained by better reporting, it most likely reflects a growing population at risk, affected by such factors as age and/or severe comorbidity. Nosocomial infections accounted for 51% of cases during the study period. The 28-day mortality, remaining unchanged at 17% throughout the 7-year follow-up, was lower than reported in previous surveys. The risk of death for nosocomial cases was almost double than that for community-acquired cases (22% vs. 13%). Interestingly, the changes in the epidemiology of SAB are very similar to those found in countries with high prevalence of MRSA.

II   Levofloxacin, combined with standard treatment, did not decrease the mortality, lower the incidence of deep infections, nor did it speed up the recovery in SAB. However, mortality for patients with deep infection was significantly lower among those who received rifampicin as compared to those treated without rifampicin (17% vs. 38%), as suggested earlier by experimental data and some small clinical trials. Deep infections were found in 84% of SAB patients within one week after randomization, and they appeared to be more common than previously reported in other studies. Prompt identification of deep infections might improve the outcome of these patients.

III   Endocarditis was more frequently connected to SAB in addicts as has also been reported previously. Additionally, IDUs were significantly younger, had less ultimately or rapidly fatal underlying diseases or predisposing heart diseases, and their SAB was more often community-acquired as compared to nonaddicts. Right-sided involvement was diagnosed in 60% of addicts whereas 93% of nonaddicts had left-sided endocarditis. Unexpectedly, IDUs showed extracardiac deep infections, thromboembolic events and severe sepsis with the same frequency as nonaddicts. The prognosis of endocarditis was better among injection drug abusers due to their younger age and lack of underlying diseases, as suggested previously.

IV No individual *S. aureus* strain was specifically associated with endocarditis among addicts. In addition, characterization of the virulence factors did not reveal a difference between IDUs and nonaddicts. However, haemolytic properties were observed more often among IDUs without endocarditis than with endocarditis. Serological tests were not helpful in identifying patients with a deep infection. Interestingly, the initial ASTA titer was more often positive among IDUs without endocarditis than those with endocarditis. One may postulate that among addicts strains without haemolytic activity could predispose to endocarditis, whereas an antibody response against staphylolysin might give some protection from it.

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9. REFERENCES

1.   Kuikka A, Valtonen VV: Improved outcome of *Staphylococcus aureus* bacteremia. Infectious Diseases in Clinical Practice 3:282-287, 1994.

2.   Lyytikäinen O, Vuopio-Varkila J: Mikrobilääkeresistenssi. In: Iivonen J, Kela E, Kuusi M, Lyytikainen O, Ruutu P, eds. Tartuntataudit Suomessa 1995-2004. Helsinki: Kansanterveyslaitos; 2005: 45-49.

3.   Conterno LO, Wey SB, Castelo A: Risk factors for mortality in *Staphylococcus aureus* bacteremia. Infect Control Hosp Epidemiol 19:32-37, 1998.

4.   Julander I, Svanbom M: Prediction of staphylococcal etiology among patients with septicemia with or without endocarditis by multivariate statistical methods. Scand J Infect Dis 17:37-46, 1985.

5.   Watanakunakorn C, Chan SJ, Demarco DG, Palmer JA: *Staphylococcus aureus* bacteremia: significance of hyperbilirubinemia. Scand J Infect Dis 19:195-203, 1987.

6.   Soriano A, Martinez JA, Mensa J, Marco F, Almela M, Moreno-Martinez A, Sanchez F, Munoz I, Jimenez de Anta MT, Soriano E: Pathogenic significance of methicillin resistance for patients with *Staphylococcus aureus* bacteremia. Clin Infect Dis 30:368-373, 2000.

7.   Mylotte JM, Tayara A: *Staphylococcus aureus* bacteremia: predictors of 30-day mortality in a large cohort. Clin Infect Dis 31:1170-1174, 2000.

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

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

10.   Benfield T, Espersen F, Frimodt-Moller N, Jensen AG, Larsen R, Pallesen LV, Skov R, Westh H, Skinhoj P: Trends in incidence and mortality among 23962 adult cases of *Staphylococcus aureus* bacteremia from 1971 through 2000. In: Program and abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 30-November 2, 2004: L-357 abstract.

11.   Fowler VG, Jr., Sanders LL, Sexton DJ, Kong L, Marr KA, Gopal AK, Gottlieb G, McClelland RS, Corey GR: Outcome of *Staphylococcus aureus* bacteremia according to compliance with recommendations of infectious diseases specialists: experience with 244 patients. Clin Infect Dis 27:478-486, 1998.

12.   Fowler VG, Jr., Sanders LL, Kong LK, McClelland RS, Gottlieb GS, Li J, Ryan T, Sexton DJ, Roussakis G, Harrell LJ, Corey GR: Infective endocarditis due to *Staphylococcus aureus*: 59 prospectively identified cases with follow-up. Clin Infect Dis 28:106-114, 1999.

13. Johnson LB, Almoujahed MO, Ilg K, Maolood L, Khatib R: *Staphylococcus aureus* bacteremia: compliance with standard treatment, long-term outcome and predictors of relapse. Scand J Infect Dis 35:782-789, 2003.

14. Kim SH, Park WB, Lee KD, Kang CI, Kim HB, Oh MD, Kim EC, Choe KW: Outcome of *Staphylococcus aureus* bacteremia in patients with eradicable foci versus noneradicable foci. Clin Infect Dis 37:794-799, 2003.

15. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y: Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 36:53-59, 2003.

16. 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 163:2066-2072, 2003.

17. Jensen AG: Importance of focus identification in the treatment of *Staphylococcus aureus* bacteraemia. J Hosp Infect 52:29-36, 2002.

18. Miro JM, Anguera I, Cabell CH, Chen AY, Stafford JA, Corey GR, Olaison L, Eykyn S, Hoen B, Abrutyn E, Raoult D, Bayer A, Fowler VG, Jr.: *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. Clin Infect Dis 41:507-514, 2005.

19. Chambers HF, Korzeniowski OM, Sande MA: *Staphylococcus aureus* endocarditis: clinical manifestations in addicts and nonaddicts. Medicine (Baltimore) 62:170-177, 1983.

20. Faber M, Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl V: *Staphylococcus aureus* endocarditis in Danish intravenous drug users: high proportion of left-sided endocarditis. Scand J Infect Dis 27:483-487, 1995.

21. Fowler VG, Jr., Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, Pappas PA, Anstrom KJ, Wray D, Fortes CQ, Anguera I, Athan E, Jones P, van der Meer JT, Elliott TS, Levine DP, Bayer AS: *Staphylococcus aureus* endocarditis: a consequence of medical progress. Jama 293:3012-3021, 2005.

22. Moreillon P, Que YA: Infective endocarditis. Lancet 363:139-49, 2004.

23. Turnidge J, Grayson ML: Optimum treatment of staphylococcal infections. Drugs 45:353-366, 1993.

24. Rayner C, Munckhof WJ: Antibiotics currently used in the treatment of infections caused by *Staphylococcus aureus*. Intern Med J 35 Suppl 2:S3-16, 2005.

25. Schmidt-Ioanas M, de Roux A, Lode H: New antibiotics for the treatment of severe staphylococcal infection in the critically ill patient. Curr Opin Crit Care 11:481-486, 2005.

26. Mitchell DH, Howden BP: Diagnosis and management of *Staphylococcus aureus* bacteraemia. Intern Med J 35 Suppl 2:S17-24, 2005.

27. Baddour LM, Wilson WR, Bayer AS, Fowler VG, Jr., Bolger AF, Levison ME, Ferrieri P, Gerber MA, Tani LY, Gewitz MH, Tong DC, Steckelberg JM, Baltimore RS, Shulman ST, Burns JC, Falace DA, Newburger JW, Pallasch TJ, Takahashi M, Taubert KA: Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation 111:e394-434, 2005.

28. Lowy FD: *Staphylococcus aureus* infections. N Engl J Med 339:520-532, 1998.

29. Van der Auwera P, Klastersky J: In vitro study of the combination of rifampin with oxacillin against *Staphylococcus aureus*. Rev Infect Dis 5 Suppl 3:S509-514, 1983.

30. Van der Auwera P, Klastersky J, Thys JP, Meunier-Carpentier F, Legrand JC: Double-blind, placebo-controlled study of oxacillin combined with rifampin in the treatment of staphylococcal infections. Antimicrob Agents Chemother 28:467-472, 1985.

31. Van der Auwera P, Meunier-Carpentier F, Klastersky J: Clinical study of combination therapy with oxacillin and rifampin for staphylococcal infections. Rev Infect Dis 5 Suppl 3:S515-522, 1983.

32. Tan JS, File TM, Jr.: Management of staphylococcal and streptococcal infections. Clin Podiatr Med Surg 13:793-816, 1996.

33. Calfee DP: Rifamycins. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett`s Principles and Practice of Infectious Diseases. 6th ed. Vol. 1. Philadelphia: Churchill Livingstone, 2005: 374-387.

34. Craig WA: Rifampin and related drugs. In: Gorbach SL, Bartlett JG, Blacklow NR, eds. Infectious Diseases. 3rd ed. Lippincott Williams & Wilkins: Philadelphia; 2004: 277-280.

35. Kapusnik JE, Parenti F, Sande MA: The use of rifampicin in staphylococcal infections - a review. J Antimicrob Chemother 13 Suppl C:61-66, 1984.

36. Hurst M, Lamb HM, Scott LJ, Figgitt DP: Levofloxacin: an updated review of its use in the treatment of bacterial infections. Drugs 62:2127-2167, 2002.

37. O'Donnell JA, Gelone SP: Fluoroquinolones. Infect Dis Clin North Am 14:489-513, xi, 2000.

38. Rohner P, Herter C, Auckenthaler R, Pechere JC, Waldvogel FA, Lew DP: Synergistic effect of quinolones and oxacillin on methicillin-resistant Staphylococcus species. Antimicrob Agents Chemother 33:2037-2041, 1989.

39. Libman H, Arbeit RD: Complications associated with *Staphylococcus aureus* bacteremia. Arch Intern Med 144:541-545, 1984.

40. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M: Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis 32 Suppl 2:S114-132, 2001.

41. Devlin RK, Andrews MM, von Reyn CF: Recent trends in infective endocarditis: influence of case definitions. Curr Opin Cardiol 19:134-139, 2004.

42. McBean M, Rajamani S: Increasing rates of hospitalization due to septicemia in the US elderly population, 1986-1997. J Infect Dis 183:596-603, 2001.

43. Wisplinghoff H, Seifert H, Coimbra M, Wenzel RP, Edmond MB: Systemic inflammatory response syndrome in adult patients with nosocomial bloodstream infection due to *Staphylococcus aureus*. Clin Infect Dis 33:733-736, 2001.

44. Steinberg JP, Clark CC, Hackman BO: Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin Infect Dis 23:255-259, 1996.

45. McClelland RS, Fowler VG, Jr., Sanders LL, Gottlieb G, Kong LK, Sexton DJ, Schmader K, Lanclos KD, Corey R: *Staphylococcus aureus* bacteremia among elderly vs younger adult patients: comparison of clinical features and mortality. Arch Intern Med 159:1244-1247, 1999.

46. Jensen AG, Wachmann CH, Poulsen KB, Espersen F, Scheibel J, Skinhoj P, Frimodt-Moller N: Risk factors for hospital-acquired *Staphylococcus aureus* bacteremia. Arch Intern Med 159:1437-1444, 1999.

47. Collignon P, Nimmo GR, Gottlieb T, Gosbell IB: *Staphylococcus aureus* bacteremia, Australia. Emerg Infect Dis 11:554-561, 2005.

48. Espersen F, Rosdahl VT, Frimodt-Mlller N, Skinhoj P: Epidemiology of *Staphylococcus aureus* bacteremia in Denmark. J Chemother 6:219-225, 1994.

49. Griffiths C, Lamagni TL, Crowcroft NS, Duckworth G, Rooney C: Trends in MRSA in England and Wales: analysis of morbidity and mortality data for 1993-2002. Health Stat Q:15-22, 2004.

50. Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT: Epidemiology of *Staphylococcus aureus* bacteremia in Denmark from 1957 to 1990. Clin Microbiol Infect 3:297-305, 1997.

51. Laupland KB, Church DL, Mucenski M, Sutherland LR, Davies HD: Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. J Infect Dis 187:1452-1459, 2003.

52. Morin CA, Hadler JL: Population-based incidence and characteristics of community-onset *Staphylococcus aureus* infections with bacteremia in 4 metropolitan Connecticut areas, 1998. J Infect Dis 184:1029-1034, 2001.

53. Morgan M, Salmon R, Keppie N, Evans-Williams D, Hosein I, Looker DN: All Wales surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA): the first year's results. J Hosp Infect 41:173-179, 1999.

54. McDonald P, Mitchell E, Johnson H, Rossney A, Humphreys H, Glynn G, Burd M, Doyle D, Mc Donnell R: MRSA bacteraemia: North/South Study of MRSA in Ireland 1999. J Hosp Infect 52:288-291, 2002.

55. Diekema DJ, Pfaller MA, Jones RN: Age-related trends in pathogen frequency and antimicrobial susceptibility of bloodstream isolates in North America: SENTRY Antimicrobial Surveillance Program, 1997-2000. Int J Antimicrob Agents 20:412-418, 2002.

56. Reacher MH, Shah A, Livermore DM, Wale MC, Graham C, Johnson AP, Heine H, Monnickendam MA, Barker KF, James D, George RC: Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. Bmj 320:213-216, 2000.

57. Cecchi E, Forno D, Imazio M, Migliardi A, Gnavi R, Dal Conte I, Trinchero R: New trends in the epidemiological and clinical features of infective endocarditis: results of a multicenter prospective study. Ital Heart J 5:249-256, 2004.

58. Cabell CH, Jollis JG, Peterson GE, Corey GR, Anderson DJ, Sexton DJ, Woods CW, Reller LB, Ryan T, Fowler VG, Jr.: Changing patient characteristics and the effect on mortality in endocarditis. Arch Intern Med 162:90-94, 2002.

59. Hoen B, Alla F, Selton-Suty C, Beguinot I, Bouvet A, Briancon S, Casalta JP, Danchin N, Delahaye F, Etienne J, Le Moing V, Leport C, Mainardi JL, Ruimy R, Vandenesch F: Changing profile of infective endocarditis: results of a 1-year survey in France. Jama 288:75-81, 2002.

60. Roder BL, Wandall DA, Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT: Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. Arch Intern Med 159:462-469, 1999.

61. Tleyjeh IM, Steckelberg JM, Murad HS, Anavekar NS, Ghomrawi HM, Mirzoyev Z, Moustafa SE, Hoskin TL, Mandrekar JN, Wilson WR, Baddour LM: Temporal trends in infective endocarditis: a population-based study in Olmsted County, Minnesota. Jama 293:3022-3028, 2005.

62. Murray RJ: *Staphylococcus aureus* infective endocarditis: diagnosis and management guidelines. Intern Med J 35 Suppl 2:S25-44, 2005.

63. Bassetti S, Battegay M: *Staphylococcus aureus* infections in injection drug users: risk factors and prevention strategies. Infection 32:163-169, 2004.

64. Mathew J, Addai T, Anand A, Morrobel A, Maheshwari P, Freels S: Clinical features, site of involvement, bacteriologic findings, and outcome of infective endocarditis in intravenous drug users. Arch Intern Med 155:1641-1648, 1995.

65.   Chang FY, MacDonald BB, Peacock JE, Jr., Musher DM, Triplett P, Mylotte JM, O'Donnell A, Wagener MM, Yu VL: A prospective multicenter study of *Staphylococcus aureus* bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance. Medicine (Baltimore) 82:322-332, 2003.

66.   Hedstrom SA, Christensson B: *Staphylococcus aureus* septicaemia and endocarditis at the University Hospital in Lund 1976-1980. Scand J Infect Dis Suppl 41:38-48, 1983.

67.   Mirimanoff RO, Glauser MP: Endocarditis during *Staphylococcus aureus* septicemia in a population of non-drug addicts. Arch Intern Med 142:1311-1313, 1982.

68.   Nadji G, Remadi JP, Coviaux F, Mirode AA, Brahim A, Enriquez-Sarano M, Tribouilloy C: Comparison of clinical and morphological characteristics of *Staphylococcus aureus* endocarditis with endocarditis caused by other pathogens. Heart 91:932-937, 2005.

69.   Bayer AS, Lam K, Ginzton L, Norman DC, Chiu CY, Ward JI: *Staphylococcus aureus* bacteremia. Clinical, serologic, and echocardiographic findings in patients with and without endocarditis. Arch Intern Med 147:457-462, 1987.

70.   Crane LR, Levine DP, Zervos MJ, Cummings G: Bacteremia in narcotic addicts at the Detroit Medical Center. I. Microbiology, epidemiology, risk factors, and empiric therapy. Rev Infect Dis 8:364-373, 1986.

71.   Levine DP, Crane LR, Zervos MJ: Bacteremia in narcotic addicts at the Detroit Medical Center. II. Infectious endocarditis: a prospective comparative study. Rev Infect Dis 8:374-396, 1986.

72.   Watanakunakorn C, Baird IM: Prognostic factors in *Staphylococcus aureus* endocarditis and results of therapy with a penicillin and gentamicin. Am J Med Sci 273:133-9, 1977.

73.   Bayer AS: Revised diagnostic criteria for infective endocarditis. Cardiol Clin 14:345-350, 1996.

74.   Bayer AS, Ward JI, Ginzton LE, Shapiro SM: Evaluation of new clinical criteria for the diagnosis of infective endocarditis. Am J Med 96:211-219, 1994.

75.   Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Jr., Ryan T, Bashore T, Corey GR: Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 30:633-638, 2000.

76.   Petti CA, Fowler VG, Jr.: *Staphylococcus aureus* bacteremia and endocarditis. Infect Dis Clin North Am 16:413-435, 2002.

77.   Banerjee SN, Emori TG, Culver DH, Gaynes RP, Jarvis WR, Horan T, Edwards JR, Tolson J, Henderson T, Martone WJ: Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. Am J Med 91:86S-89S, 1991.

78.   Karchmer AW: Nosocomial bloodstream infections: organisms, risk factors, and implications. Clin Infect Dis 31 Suppl 4:S139-143, 2000.

79.   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 40:695-703, 2005.

80.   McGowan JE, Jr., Barnes MW, Finland M: Bacteremia at Boston City Hospital: Occurrence and mortality during 12 selected years (1935-1972), with special reference to hospital-acquired cases. J Infect Dis 132:316-335, 1975.

81.   Crowe M, Ispahani P, Humphreys H, Kelley T, Winter R: Bacteraemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, 1985-1996. Eur J Clin Microbiol Infect Dis 17:377-384, 1998.

82.   Finkelstein R, Sobel JD, Nagler A, Merzbach D: *Staphylococcus aureus* bacteremia and endocarditis: comparison of nosocomial and community-acquired infection. J Med 15:193-211, 1984.

83.   Cunney RJ, McNamara EB, alAnsari N, Smyth EG: Community and hospital acquired *Staphylococcus aureus* septicaemia: 115 cases from a Dublin teaching hospital. J Infect 33:11-3, 1996.

84.   Lautenschlager S, Herzog C, Zimmerli W: Course and outcome of bacteremia due to *Staphylococcus aureus*: evaluation of different clinical case definitions. Clin Infect Dis 16:567-573, 1993.

85.   Maradona JA, Carton JA, Lopez-Alonso J, Carcaba V, Nuno FJ, Arribas JM: Comparative study of community versus hospital-acquired *Staphylococcus aureus* bacteraemia. Eur J Med 1:113-115, 1992.

86.   Moreillon P, Que YA, Glauser MP: *Staphylococcus aureus* (including staphylococcal toxic shock). In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett`s Principles and Practice of Infectious Diseases. 6th ed. Vol. 2. Philadelphia: Churchill Livingstone, 2005: 2321-2351.

87.   Ibrahem S, Salmenlinna S, Kerttula AM, Virolainen-Julkunen A, Kuusela P, Vuopio-Varkila J: Comparison of genotypes of methicillin-resistant and methicillin-*sensitive Staphylococcus aureus* in Finland. Eur J Clin Microbiol Infect Dis 24:325-328, 2005.

88.   Monnet DL, Frimodt-Moller N: Antimicrobial-drug use and methicillin-resistant *Staphylo-coccus aureus*. Emerg Infect Dis 7:161-163, 2001.

89.   Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundman H: Methicillin-resistant *Staphylococcus aureus* in Europe, 1999-2002. Emerg Infect Dis 10:1627-1634, 2004.

90.   Boyce JM, Cookson B, Christiansen K, Hori S, Vuopio-Varkila J, Kocagoz S, Oztop AY, Vandenbroucke-Grauls CM, Harbarth S, Pittet D: Meticillin-resistant *Staphylococcus aureus*. Lancet Infect Dis 5:653-663, 2005.

91. Lyytikäinen O, Lumio J, Sarkkinen H, Kolho E, Kostiala A, Ruutu P: Nosocomial bloodstream infections in Finnish hospitals during 1999-2000. Clin Infect Dis 35:e14-19, 2002.

92. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ: Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. Infect Control Hosp Epidemiol 13:582-586, 1992.

93. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R: Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin Infect Dis 42:389-391, 2006.

94. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piemont Y, Brousse N, Floret D, Etienne J: Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359:753-759, 2002.

95. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R: Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290:2976-2984, 2003.

96. Salgado CD, Farr BM, Calfee DP: Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin Infect Dis 36:131-139, 2003.

97. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM: Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 352:1436-1444, 2005.

98. Kluytmans-Vandenbergh MF, Kluytmans JA: Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. Clin Microbiol Infect 12 Suppl 1:9-15, 2006.

99. Maltezou HC, Giamarellou H: Community-acquired methicillin-resistant *Staphylococcus aureus* infections. Int J Antimicrob Agents 27:87-96, 2006.

100. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I: Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 350:1670-1673, 1997.

101. Appelbaum PC: The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12 Suppl 1:16-23, 2006.

102. Fridkin SK, Edwards JR, Tenover FC, Gaynes RP, McGowan JE, Jr.: Antimicrobial resistance prevalence rates in hospital antibiograms reflect prevalence rates among pathogens associated with hospital-acquired infections. Clin Infect Dis 33:324-330, 2001.

103.   Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR: Emergence of vancomycin resistance in *Staphylococcus aureus*. Glycopeptide-Intermediate *Staphylococcus* aureus Working Group. N Engl J Med 340:493-501, 1999.

104.   Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, Shah S, Rudrik JT, Pupp GR, Brown WJ, Cardo D, Fridkin SK: Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. N Engl J Med 348:1342-1347, 2003.

105.   Tenover FC, Weigel LM, Appelbaum PC, McDougal LK, Chaitram J, McAllister S, Clark N, Killgore G, O'Hara CM, Jevitt L, Patel JB, Bozdogan B: Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrob Agents Chemother 48:275-280, 2004.

106.   Kluytmans J, van Belkum A, Verbrugh H: Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10:505-520, 1997.

107.   Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA: Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364:703-705, 2004.

108.   Lipsky BA, Pecoraro RE, Chen MS, Koepsell TD: Factors affecting staphylococcal colonization among NIDDM outpatients. Diabetes Care 10:483-486, 1987.

109.   Yu VL, Goetz A, Wagener M, Smith PB, Rihs JD, Hanchett J, Zuravleff JJ: *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis. Efficacy of antibiotic prophylaxis. N Engl J Med 315:91-96, 1986.

110.   Hoeger PH, Lenz W, Boutonnier A, Fournier JM: Staphylococcal skin colonization in children with atopic dermatitis: prevalence, persistence, and transmission of toxigenic and nontoxigenic strains. J Infect Dis 165:1064-1068, 1992.

111.   Tuazon CU, Sheagren JN: Increased rate of carriage of *Staphylococcus aureus* among narcotic addicts. J Infect Dis 129:725-727, 1974.

112.   Miller M, Cespedes C, Vavagiakis P, Klein RS, Lowy FD: *Staphylococcus aureus* colonization in a community sample of HIV-infected and HIV-uninfected drug users. Eur J Clin Microbiol Infect Dis 22:463-469, 2003.

113.   Lowy FD, Miller M: New methods to investigate infectious disease transmission and pathogenesis - *Staphylococcus aureus* disease in drug users. Lancet Infect Dis 2:605-612, 2002.

114.   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 344:11-16, 2001.

115. Kluytmans JA, Manders MJ, van Bommel E, Verbrugh H: Elimination of nasal carriage of *Staphylococcus aureus* in hemodialysis patients. Infect Control Hosp Epidemiol 17:793-797, 1996.

116. Boelaert JR, De Baere YA, Geernaert MA, Godard CA, Van Landuyt HW: The use of nasal mupirocin ointment to prevent *Staphylococcus aureus* bacteraemias in haemodialysis patients: an analysis of cost-effectiveness. J Hosp Infect 19 Suppl B:41-46, 1991.

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

118. Wertheim HF, Vos MC, Ott A, Voss A, Kluytmans JA, Vandenbroucke-Grauls CM, Meester MH, van Keulen PH, Verbrugh HA: Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. Ann Intern Med 140:419-425, 2004.

119. Espersen F: Identifying the patient risk for *Staphylococcus aureus* bloodstream infections. J Chemother 7 Suppl 3:11-17, 1995.

120. Jensen AG: *Staphylococcus aureus* bacteremia. Dan Med Bull 50:423-438, 2003.

121. Mylotte JM, McDermott C, Spooner JA: Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. Rev Infect Dis 9:891-907, 1987.

122. Gransden WR, Eykyn SJ, Phillips I: *Staphylococcus aureus* bacteraemia: 400 episodes in St Thomas's Hospital. Br Med J (Clin Res Ed) 288:300-303, 1984.

123. Nolan CM, Beaty HN: *Staphylococcus aureus* bacteremia. Current clinical patterns. Am J Med 60:495-500, 1976.

124. Espersen F, Frimodt-Moller N: *Staphylococcus aureus* endocarditis. A review of 119 cases. Arch Intern Med 146:1118-1121, 1986.

125. Abraham J, Mansour C, Veledar E, Khan B, Lerakis S: *Staphylococcus aureus* bacteremia and endocarditis: the Grady Memorial Hospital experience with methicillin-sensitive S aureus and methicillin-resistant S aureus bacteremia. Am Heart J 147:536-539, 2004.

126. Mylonakis E, Calderwood SB: Infective endocarditis in adults. N Engl J Med 345:1318-1330, 2001.

127. Cabell CH, Fowler VG, Jr.: Importance of aggressive evaluation in patients with *Staphylococcus aureus* bacteremia. Am Heart J 147:379-380, 2004.

128. Lesens O, Hansmann Y, Storck D, Christmann D: Risk factors for metastatic infection in patients with *Staphylococcus aureus* bacteremia with and without endocarditis. Eur J Intern Med 14:227-231, 2003.

129. Blyth CC, Darragh H, Whelan A, O'Shea JP, Beaman MH, McCarthy JS: Evaluation of clinical guidelines for the management of *Staphylococcus aureus* bacteraemia. Intern Med J 32:224-232, 2002.

130. Marr KA, Kong L, Fowler VG, Gopal A, Sexton DJ, Conlon PJ, Corey GR: Incidence and outcome of *Staphylococcus aureus* bacteremia in hemodialysis patients. Kidney Int 54:1684-1689, 1998.

131. Khatib R, Johnson LB, Fakih MG, Riederer K, Khosrovaneh A, Shamse Tabriz M, Sharma M, Saeed S: Persistence in *Staphylococcus aureus* bacteremia: Incidence, characteristics of patients and outcome. Scand J Infect Dis 38:7-14, 2006.

132. Lesens O, Hansmann Y, Brannigan E, Remy V, Hopkins S, Martinot M, Meyer P, Connel BO, Monteil H, Christmann D, Bergin C: Positive surveillance blood culture is a predictive factor for secondary metastatic infection in patients with *Staphylococcus aureus* bacteraemia. J Infect 48:245-252, 2004.

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

134. Romero-Vivas J, Rubio M, Fernandez C, Picazo JJ: Mortality associated with nosocomial bacteremia due to methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 21:1417-1423, 1995.

135. Harbarth S, Rutschmann O, Sudre P, Pittet D: Impact of methicillin resistance on the outcome of patients with bacteremia caused by *Staphylococcus aureus*. Arch Intern Med 158:182-189, 1998.

136. Small PM, Chambers HF: Vancomycin for *Staphylococcus aureus* endocarditis in intravenous drug users. Antimicrob Agents Chemother 34:1227-1231, 1990.

137. Levine DP, Fromm BS, Reddy BR: Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. Ann Intern Med 115:674-680, 1991.

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

139. Muder RR, Brennen C, Wagener MM, Goetz AM: Bacteremia in a long-term-care facility: a five-year prospective study of 163 consecutive episodes. Clin Infect Dis 14:647-654, 1992.

140. Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, Stout JE, Yu VL: Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. Clin Infect Dis 42:46-50, 2006.

141. Lee BK, Crossley K, Gerding DN: The association between *Staphylococcus aureus* bacteremia and bacteriuria. Am J Med 65:303-306, 1978.

142.   Murdoch DR, Roberts SA, Fowler Jr VG, Jr., Shah MA, Taylor SL, Morris AJ, Corey GR: Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. Clin Infect Dis 32:647-649, 2001.

143.   Willcox PA, Rayner BL, Whitelaw DA: Community-acquired *Staphylococcus aureus* bacteraemia in patients who do not abuse intravenous drugs. Qjm 91:41-47, 1998.

144.   Hill PC, Birch M, Chambers S, Drinkovic D, Ellis-Pegler RB, Everts R, Murdoch D, Pottumarthy S, Roberts SA, Swager C, Taylor SL, Thomas MG, Wong CG, Morris AJ: Prospective study of 424 cases of *Staphylococcus aureus* bacteraemia: determination of factors affecting incidence and mortality. Intern Med J 31:97-103, 2001.

145.   Ringberg H, Thoren A, Lilja B: Metastatic complications of *Staphylococcus aureus* septicemia. To seek is to find. Infection 28:132-136, 2000.

146.   Bamberger DM, Boyd SE: Management of *Staphylococcus aureus* infections. Am Fam Physician 72:2474-2481, 2005.

147.   Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, Wade JC: Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis 41:1373-1406, 2005.

148.   Brennan TA, Leape LL, Laird NM, Hebert L, Localio AR, Lawthers AG, Newhouse JP, Weiler PC, Hiatt HH: Incidence of adverse events and negligence in hospitalized patients. Results of the Harvard Medical Practice Study I. N Engl J Med 324:370-376, 1991.

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

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

151.   Jernigan JA, Farr BM: Short-course therapy of catheter-related *Staphylococcus aureus* bacteremia: a meta-analysis. Ann Intern Med 119:304-311, 1993.

152.   Raad, II, Sabbagh MF: Optimal duration of therapy for catheter-related *Staphylococcus aureus* bacteremia: a study of 55 cases and review. Clin Infect Dis 14:75-82, 1992.

153.   Fowler VG, Jr., Li J, Corey GR, Boley J, Marr KA, Gopal AK, Kong LK, Gottlieb G, Donovan CL, Sexton DJ, Ryan T: Role of echocardiography in evaluation of patients with *Staphylococcus aureus* bacteremia: experience in 103 patients. J Am Coll Cardiol 30:1072-1078, 1997.

154.   Wilson R, Hamburger M: Fifteen years' experience with staphylococcus septicemia in a large city hospital; analysis of fifty-five cases in the Cincinnati General Hospital 1940 to 1954. Am J Med 22:437-457, 1957.

155.   Von Reyn CF, Levy BS, Arbeit RD, Friedland G, Crumpacker CS: Infective endocarditis: an analysis based on strict case definitions. Ann Intern Med 94:505-518, 1981.

156. Durack DT, Lukes AS, Bright DK: New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. Am J Med 96:200-209, 1994.

157. Karchmer AW: Infective endocarditis. In: Braunwald E, Zipes DP, Libby P, eds. Heart Disease: A Textbook of Cardiovascular Medicine. 6th ed. W.B. Saunders Company: Philadelphia, 2001: 1723-1750.

158. Chirillo F, Pedrocco A, De Leo A, Bruni A, Totis O, Meneghetti P, Stritoni P: Impact of harmonic imaging on transthoracic echocardiographic identification of infective endocarditis and its complications. Heart 91:329-333, 2005.

159. Shively BK, Gurule FT, Roldan CA, Leggett JH, Schiller NB: Diagnostic value of transesophageal compared with transthoracic echocardiography in infective endocarditis. J Am Coll Cardiol 18:391-397, 1991.

160. Werner GS, Schulz R, Fuchs JB, Andreas S, Prange H, Ruschewski W, Kreuzer H: Infective endocarditis in the elderly in the era of transesophageal echocardiography: clinical features and prognosis compared with younger patients. Am J Med 100:90-97, 1996.

161. Rosen AB, Fowler VG, Jr., Corey GR, Downs SM, Biddle AK, Li J, Jollis JG: Cost-effectiveness of transesophageal echocardiography to determine the duration of therapy for intravascular catheter-associated *Staphylococcus aureus* bacteremia. Ann Intern Med 130:810-820, 1999.

162. Sjursen H: In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 210-236.

163. Lien EA, Solberg CO, Kalager T: Infective endocarditis 1973-1984 at the Bergen University Hospital: clinical feature, treatment and prognosis. Scand J Infect Dis 20:239-246, 1988.

164. Fowler VG, Jr., Schelenz S, Bayer AS: Endocarditis and intravascular infections. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett`s Principles and Practice of Infectious Diseases. 6th ed. Vol. 2. Philadelphia: Churchill Livingstone, 2005: 75-1021.

165. Steckelberg JM, Murphy JG, Ballard D, Bailey K, Tajik AJ, Taliercio CP, Giuliani ER, Wilson WR: Emboli in infective endocarditis: the prognostic value of echocardiography. Ann Intern Med 114:635-640, 1991.

166. Vilacosta I, Graupner C, San Roman JA, Sarria C, Ronderos R, Fernandez C, Mancini L, Sanz O, Sanmartin JV, Stoermann W: Risk of embolization after institution of antibiotic therapy for infective endocarditis. J Am Coll Cardiol 39:1489-1495, 2002.

167. Roder BL, Wandall DA, Espersen F, Frimodt-Moller N, Skinhoj P, Rosdahl VT: Neurologic manifestations in *Staphylococcus aureus* endocarditis: a review of 260 bacteremic cases in nondrug addicts. Am J Med 102:379-386, 1997.

168. Thuny F, Disalvo G, Belliard O, Avierinos JF, Pergola V, Rosenberg V, Casalta JP, Gouvernet J, Derumeaux G, Iarussi D, Ambrosi P, Calabro R, Riberi A, Collart F, Metras D, Lepidi H, Raoult D, Harle JR, Weiller PJ, Cohen A, Habib G: Risk of embolism and death in infective endocarditis: prognostic value of echocardiography: a prospective multicenter study. Circulation 112:69-75, 2005.

169. Hasbun R, Vikram HR, Barakat LA, Buenconsejo J, Quagliarello VJ: Complicated left-sided native valve endocarditis in adults: risk classification for mortality. JAMA 289:1933-1940, 2003.

170. Baddour LM, Bisno AL: Infective endocarditis complicating mitral valve prolapse: epidemiologic, clinical, and microbiologic aspects. Rev Infect Dis 8:117-1137, 1986.

171. Chamis AL, Peterson GE, Cabell CH, Corey GR, Sorrentino RA, Greenfield RA, Ryan T, Reller LB, Fowler VG, Jr.: *Staphylococcus aureus* bacteremia in patients with permanent pacemakers or implantable cardioverter-defibrillators. Circulation 104:1029-1033, 2001.

172. Fabri J, Jr., Issa VS, Pomerantzeff PM, Grinberg M, Barretto AC, Mansur AJ: Time-related distribution, risk factors and prognostic influence of embolism in patients with left-sided infective endocarditis. Int J Cardiol 110:334-339, 2006.

173. Heiro M, Nikoskelainen J, Engblom E, Kotilainen E, Marttila R, Kotilainen P: Neurologic manifestations of infective endocarditis: a 17-year experience in a teaching hospital in Finland. Arch Intern Med 160:2781-2787, 2000.

174. El-Ahdab F, Benjamin DK, Jr., Wang A, Cabell CH, Chu VH, Stryjewski ME, Corey GR, Sexton DJ, Reller LB, Fowler VG, Jr.: Risk of endocarditis among patients with prosthetic valves and *Staphylococcus aureus* bacteremia. Am J Med 118:225-229, 2005.

175. Fang G, Keys TF, Gentry LO, Harris AA, Rivera N, Getz K, Fuchs PC, Gustafson M, Wong ES, Goetz A, Wagener MM, Yu VL: Prosthetic valve endocarditis resulting from nosocomial bacteremia. A prospective, multicenter study. Ann Intern Med 119:560-567, 1993.

176. Ribera E, Gomez-Jimenez J, Cortes E, del Valle O, Planes A, Gonzalez-Alujas T, Almirante B, Ocana I, Pahissa A: Effectiveness of cloxacillin with and without gentamicin in short-term therapy for right-sided *Staphylococcus aureus* endocarditis. A randomized, controlled trial. Ann Intern Med 125:969-974, 1996.

177. Baddour LM, Wilson FM: Infections of prosthetic valves and other cardiovascular devices. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett`s Principles and Practice of Infectious Diseases. 6th ed. Vol. 2. Philadelphia: Churchill Livingstone, 2005: 1022-1044.

178. Gordon RJ, Lowy FD: Bacterial infections in drug users. N Engl J Med 353:1945-1954, 2005.

179. Levine DP, Brown PD: Infections in injection drug users. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett`s Principles and Practice of Infectious Diseases. 6th ed. Vol. 2. Philadelphia: Churchill Livingstone, 2005: 3462-3476.

180. Tuazon CU, Sheagren JN: Staphylococcal endocarditis in parenteral drug abusers: source of the organism. Ann Intern Med 82:788-790, 1975.

181. Craven DE, Rixinger AI, Goularte TA, McCabe WR: Methicillin-resistant *Staphylococcus aureus* bacteremia linked to intravenous drug abusers using a "shooting gallery". Am J Med 80:770-776, 1986.

182. Fleisch F, Zbinden R, Vanoli C, Ruef C: Epidemic spread of a single clone of methicillin-resistant *Staphylococcus aureus* among injection drug users in Zurich, Switzerland. Clin Infect Dis 32:581-586, 2001.

183. Quagliarello B, Cespedes C, Miller M, Toro A, Vavagiakis P, Klein RS, Lowy FD: Strains of *Staphylococcus aureus* obtained from drug-use networks are closely linked. Clin Infect Dis 35:671-677, 2002.

184. Wilson LE, Thomas DL, Astemborski J, Freedman TL, Vlahov D: Prospective study of infective endocarditis among injection drug users. J Infect Dis 185:1761-1766, 2002.

185. Hecht SR, Berger M: Right-sided endocarditis in intravenous drug users. Prognostic features in 102 episodes. Ann Intern Med 117:560-566, 1992.

186. Miro JM, del Rio A, Mestres CA: Infective endocarditis in intravenous drug abusers and HIV-1 infected patients. Infect Dis Clin North Am 16:273-295, 2002.

187. Watanakunakorn C, Burkert T: Infective endocarditis at a large community teaching hospital, 1980-1990. A review of 210 episodes. Medicine (Baltimore) 72:90-102, 1993.

188. Reisberg BE: Infective endocarditis in the narcotic addict. Prog Cardiovasc Dis 22:193-204, 1979.

189. Watanakunakorn C: *Staphylococcus aureus* endocarditis at a community teaching hospital, 1980 to 1991. An analysis of 106 cases. Arch Intern Med 154:2330-2335, 1994.

190. Frontera JA, Gradon JD: Right-side endocarditis in injection drug users: review of proposed mechanisms of pathogenesis. Clin Infect Dis 30:374-379, 2000.

191. Miro JM, Moreno A, Mestres CA: Infective endocarditis in intravenous drug abusers. Curr Infect Dis Rep 5:307-316, 2003.

192. Chua JD, Wilkoff BL, Lee I, Juratli N, Longworth DL, Gordon SM: Diagnosis and management of infections involving implantable electrophysiologic cardiac devices. Ann Intern Med 133:604-608, 2000.

193. Julander I: Staphylococcal septicaemia and endocarditis in 80 drug addicts. Aspects on epidemiology, clinical and laboratory findings and prognosis. Scand J Infect Dis Suppl 41:49-55, 1983.

194. Bouza E, Menasalvas A, Munoz P, Vasallo FJ, del Mar Moreno M, Garcia Fernandez MA: Infective endocarditis - a prospective study at the end of the twentieth century: new predisposing conditions, new etiologic agents, and still a high mortality. Medicine (Baltimore) 80:298-307, 2001.

195. Sklaver AR, Hoffman TA, Greenman RL: Staphylococcal endocarditis in addicts. South Med J 71:638-643, 1978.

196. Graves MK, Soto L: Left-sided endocarditis in parenteral drug abusers: recent experience at a large community hospital. South Med J 85:378-380, 1992.

197. Hart RG, Foster JW, Luther MF, Kanter MC: Stroke in infective endocarditis. Stroke 21:695-700, 1990.

198. Hedstrom SA: Bone and joint infections. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 250-271.

199. Torda AJ, Gottlieb T, Bradbury R: Pyogenic vertebral osteomyelitis: analysis of 20 cases and review. Clin Infect Dis 20:320-328, 1995.

200. Espersen F, Frimodt-Moller N, Thamdrup Rosdahl V, Skinhoj P, Bentzon MW: Changing pattern of bone and joint infections due to *Staphylococcus aureus*: study of cases of bacteremia in Denmark, 1959-1988. Rev Infect Dis 13:347-358, 1991.

201. Goldenberg DL, Cohen AS: Acute infectious arthritis. A review of patients with nongonococcal joint infections (with emphasis on therapy and prognosis). Am J Med 60:369-377, 1976.

202. Kim JH, van der Horst C, Mulrow CD, Corey GR: *Staphylococcus aureus* meningitis: review of 28 cases. Rev Infect Dis 11:698-706, 1989.

203. Jensen AG, Espersen F, Skinhoj P, Rosdahl VT, Frimodt-Moller N: *Staphylococcus aureus* meningitis. A review of 104 nationwide, consecutive cases. Arch Intern Med 153:1902-1908, 1993.

204. Fang GD, Fine M, Orloff J, Arisumi D, Yu VL, Kapoor W, Grayston JT, Wang SP, Kohler R, Muder RR, et al.: New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. Medicine (Baltimore) 9:307-316, 1990.

205. Kayser FH: Changes in the spectrum of organisms causing respiratory tract infections: a review. Postgrad Med J 68 Suppl 3:S17-23, 1992.

206. Miller RF, Foley NM, Kessel D, Jeffrey AA: Community acquired lobar pneumonia in patients with HIV infection and AIDS. Thorax 49:367-368, 1994.

207. Hoban D, Waites K, Felmingham D: Antimicrobial susceptibility of community-acquired respiratory tract pathogens in North America in 1999-2000: findings of the PROTEKT surveillance study. Diagn Microbiol Infect Dis 45:251-259, 2003.

208. Tuazon CU, Cardella TA, Sheagren JN: Staphylococcal endocarditis in drug users. Clinical and microbiologic aspects. Arch Intern Med 135:1555-1561, 1975.

209. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J: Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128-1132, 1999.

210. Grill AK, Domb S: Case report: *Staphylococcus aureus* bacteriuria. Important indicator of coexistent bacteremia? Can Fam Physician 50:879-880, 2004.

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

212. Musher DM, McKenzie SO: Infections due to *Staphylococcus aureus*. Medicine (Baltimore) 56:383-409, 1977.

213. Davis JS: Management of bone and joint infections due to *Staphylococcus aureus*. Intern Med J 35 Suppl 2:S79-96, 2005.

214. Kotilainen P: Foreign body infections. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 274-319.

215. Chu VH, Crosslin DR, Friedman JY, Reed SD, Cabell CH, Griffiths RI, Masselink LE, Kaye KS, Corey GR, Reller LB, Stryjewski ME, Schulman KA, Fowler VG, Jr.: *Staphylococcus aureus* bacteremia in patients with prosthetic devices: costs and outcomes. Am J Med 118:1416, 2005.

216. Skinner D, Keefer CS: Significance of bacteremia caused by *Staphylococcus aureus*. Arch Intern Med 68:851-875, 1941.

217. Faber V, Jessen O, Rosendal K, Eriksen KR: Staphylococcal bacteraemia. Clinical and bacteriological observations in 201 cases. Br Med J 5216:1832-1836, 1960.

218. Cooper G, Platt R: *Staphylococcus aureus* bacteremia in diabetic patients. Endocarditis and mortality. Am J Med 73:658-662, 1982.

219. Hassall JE, Rountree PM: Staphylococcal septicaemia. Lancet 1:213-217, 1959.

220. Mylotte JM, Beam TR, Jr., Allen JC: *Staphylococcus aureus* bacteremia: a prospective study. South Med J 76:1131-1135, 1983.

221. Bryan CS, Kirkhart B, Brenner ER: Staphylococcal bacteremia: current patterns in nonuniversity hospitals. South Med J 77:693-696, 1984.

222. Gonzalez-Barca E, Carratala J, Mykietiuk A, Fernandez-Sevilla A, Gudiol F: Predisposing factors and outcome of *Staphylococcus aureus* bacteremia in neutropenic patients with cancer. Eur J Clin Microbiol Infect Dis 20:117-119, 2001.

223. Yzerman EP, Boelens HA, Tjhie JH, Kluytmans JA, Mouton JW, Verbrugh HA: Delta APACHE II for predicting course and outcome of nosocomial *Staphylococcus aureus* bacteremia and its relation to host defense. J Infect Dis 173:914-919, 1996.

224. Julander I: Unfavourable prognostic factors in *Staphylococcus aureus* septicemia and endocarditis. Scand J Infect Dis 17:179-187, 1985.

225. Terpenning MS, Buggy BP, Kauffman CA: Infective endocarditis: clinical features in young and elderly patients. Am J Med 83:626-634, 1987.

226. Garrison PK, Freedman LR: Experimental endocarditis I. Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of the heart. Yale J Biol Med 42:394-410, 1970.

227. Perlman BB, Freedman LR: Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. Yale J Biol Med 44:206-213, 1971.

228. Torres Tortosa M, Gonzalez Serrano M, Perez Guzman E, Vergara de Campos A, Perez Jimenez J, Perez Cortes S, Gutierrez de la Pena J, Sanchez Porto A, Alvarez Alcina MM, Perez Moreno JM: Infective endocarditis in heroin addicts in the province of Cadiz. A multicenter study of 150 cases. Med Clin (Barc) 98:521-526, 1992.

229. Blot SI, Vandewoude KH, Hoste EA, Colardyn FA: Outcome and attributable mortality in critically Ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. Arch Intern Med 162:2229-2235, 2002.

230. Lodise TP, McKinnon PS: Clinical and economic impact of methicillin resistance in patients with *Staphylococcus aureus* bacteremia. Diagn Microbiol Infect Dis 52:113-122, 2005.

231. French GL, Cheng AF, Ling JM, Mo P, Donnan S: Hong Kong strains of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* have similar virulence. J Hosp Infect 15:117-125, 1990.

232. Pfaller MA, Jones RN, Doern GV, Sader HS, Kugler KC, Beach ML: Survey of blood stream infections attributable to gram-positive cocci: frequency of occurrence and antimicrobial susceptibility of isolates collected in 1997 in the United States, Canada, and Latin America from the SENTRY Antimicrobial Surveillance Program. SENTRY Participants Group. Diagn Microbiol Infect Dis 33:283-297, 1999.

233. Moise-Broder PA, Sakoulas G, Eliopoulos GM, Schentag JJ, Forrest A, Moellering RC, Jr.: Accessory gene regulator group II polymorphism in methicillin-resistant *Staphylococcus aureus* is predictive of failure of vancomycin therapy. Clin Infect Dis 38:1700-1705, 2004.

234. Fowler VG, Jr., Kong LK, Corey GR, Gottlieb GS, McClelland RS, Sexton DJ, Gesty-Palmer D, Harrell LJ: Recurrent *Staphylococcus aureus* bacteremia: pulsed-field gel electrophoresis findings in 29 patients. J Infect Dis 179:1157-1161, 1999.

235. Chang FY, Peacock JE, Jr., Musher DM, Triplett P, MacDonald BB, Mylotte JM, O'Donnell A, Wagener MM, Yu VL: *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. Medicine (Baltimore) 82:333-339, 2003.

236. Marr KA, Sexton DJ, Conlon PJ, Corey GR, Schwab SJ, Kirkland KB: Catheter-related bacteremia and outcome of attempted catheter salvage in patients undergoing hemodialysis. Ann Intern Med 127:275-280, 1997.

237. Siegman-Igra Y, Reich P, Orni-Wasserlauf R, Schwartz D, Giladi M: The role of vancomycin in the persistence or recurrence of *Staphylococcus aureus* bacteraemia. Scand J Infect Dis 37:572-578, 2005.

238. Tice AD, Hoaglund PA, Shoultz DA: Outcomes of osteomyelitis among patients treated with outpatient parenteral antimicrobial therapy. Am J Med 114:723-728, 2003.

239. Seligman SJ: Current concepts of *Staphylococcus aureus* infection with emphasis on treatment. Compr Ther 9:27-32, 1983.

240. Watanakunakorn C: Clindamycin therapy of *Staphylococcus aureus* endocarditis. Clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin. Am J Med 60:419-425, 1976.

241. Hartstein AI, Mulligan ME, Morthland VH, Kwok RY: Recurrent *Staphylococcus aureus* bacteremia. J Clin Microbiol 30:670-674, 1992.

242. Mortara LA, Bayer AS: *Staphylococcus aureus* bacteremia and endocarditis. New diagnostic and therapeutic concepts. Infect Dis Clin North Am 7:53-68, 1993.

243. Mylotte JM, Aeschlimann JR, Rotella DL: *Staphylococcus aureus* bacteremia: factors predicting hospital mortality. Infect Control Hosp Epidemiol 17:165-168, 1996.

244. Darouiche RO: Treatment of infections associated with surgical implants. N Engl J Med 350:1422-1429, 2004.

245. Ruiz ME, Guerrero IC, Tuazon CU: Endocarditis caused by methicillin-resistant *Staphylococcus aureus*: treatment failure with linezolid. Clin Infect Dis 35:1018-1020, 2002.

246. Falagas ME, Manta KG, Ntziora F, Vardakas KZ: Linezolid for the treatment of patients with endocarditis: a systematic review of the published evidence. J Antimicrob Chemother 58:273-280, 2006.

247. Malanoski GJ, Samore MH, Pefanis A, Karchmer AW: *Staphylococcus aureus* catheter-associated bacteremia. Minimal effective therapy and unusual infectious complications associated with arterial sheath catheters. Arch Intern Med 155:1161-1166, 1995.

248. Kim AI, Adal KA, Schmitt SK: *Staphylococcus aureus* bacteremia: using echocardiography to guide length of therapy. Cleve Clin J Med 70:517, 520-521, 525-526 passim, 2003.

249. Watanakunakorn C: Increasing importance of intravascular device-associated *Staphylococcus aureus* endocarditis. Clin Infect Dis 28:115-116, 1999.

250. Rahal JJ, Jr., Chan YK, Johnson G: Relationship of staphylococcal tolerance, teichoic acid antibody, and serum bactericidal activity to therapeutic outcome in *Staphylococcus aureus* bacteremia. Am J Med 81:43-52, 1986.

251. Chang FY: *Staphylococcus aureus* bacteremia and endocarditis. J Microbiol Immunol Infect 33:63-68, 2000.

252. Miller MH, Wexler MA, Steigbigel NH: Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. Antimicrob Agents Chemother 14:336-343, 1978.

253. Le T, Bayer AS: Combination antibiotic therapy for infective endocarditis. Clin Infect Dis 36:615-621, 2003.

254. Andriole VT: Staphylococci and combination therapy. Arch Intern Med 139:1090-1091, 1979.

255. Svanbom M: Antibiotic treatment of staphylococcal septicaemia and endocarditis in a Swedish hospital. Scand J Infect Dis Suppl 41:161-172, 1983.

256. Solberg CO: Spread of *Staphylococcus aureus* in hospitals: causes and prevention. Scand J Infect Dis 32:587-595, 2000.

257. Korzeniowski O, Sande MA: Combination antimicrobial therapy for *Staphylococcus aureus* endocarditis in patients addicted to parenteral drugs and in nonaddicts: A prospective study. Ann Intern Med 97:496-503, 1982.

258. Sabath LD, Garner C, Wilcox C, Finland M: Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 antibiotics. Antimicrob Agents Chemother 9:962-969, 1976.

259. Krut O, Sommer H, Kronke M: Antibiotic-induced persistence of cytotoxic *Staphylococcus aureus* in non-phagocytic cells. J Antimicrob Chemother 53:167-173, 2004.

260. Chambers HF, Xiang Q, Liu, Chow LL, Hackbarth C: Efficacy of levofloxacin for experimental aortic-valve endocarditis in rabbits infected with viridans group streptococcus or *Staphylococcus aureus*. Antimicrob Agents Chemother 43:2742-2746, 1999.

261. Kaatz GW, Seo SM, Barriere SL, Albrecht LM, Rybak MJ: Ciprofloxacin and rifampin, alone and in combination, for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob Agents Chemother 33:1184-1187, 1989.

262. Dworkin R, Modin G, Kunz S, Rich R, Zak O, Sande M: Comparative efficacies of ciprofloxacin, pefloxacin, and vancomycin in combination with rifampin in a rat model of methicillin-resistant *Staphylococcus aureus* chronic osteomyelitis. Antimicrob Agents Chemother 34:1014-1016, 1990.

263. Norden CW, Shaffer M: Treatment of experimental chronic osteomyelitis due to *Staphylococcus aureus* with vancomycin and rifampin. J Infect Dis 147:352-357, 1983.

264. Acar JF, Goldstein FW, Duval J: Use of rifampin for the treatment of serious staphylococcal and gram-negative bacillary infections. Rev Infect Dis 5 Suppl 3:S502-506, 1983.

265. Van der Auwera P, Thys JP, Meunier-Carpentier F, Klastersky J: The combination of oxacillin with rifampicin in staphylococcal infections: a review of laboratory and clinical studies of the Institut Jules Bordet. J Antimicrob Chemother 13 Suppl C:31-42, 1984.

266. Zinner SH, Lagast H, Klastersky J: Antistaphylococcal activity of rifampin with other antibiotics. J Infect Dis 144:365-371, 1981.

267. Zavasky DM, Sande MA: Reconsideration of rifampin: a unique drug for a unique infection. Jama 279:1575-1577, 1998.

268. Norden CW, Fierer J, Bryant RE: Chronic staphylococcal osteomyelitis: treatment with regimens containing rifampin. Rev Infect Dis 5 Suppl 3:S495-501, 1983.

269. Norden CW, Bryant R, Palmer D, Montgomerie JZ, Wheat J: Chronic osteomyelitis caused by *Staphylococcus aureus*: controlled clinical trial of nafcillin therapy and nafcillin-rifampin therapy. South Med J 79:947-951, 1986.

270. Andriole VT: The quinolones: past, present, and future. Clin Infect Dis 41 Suppl 2:S113-119, 2005.

271. Smith RP, Baltch AL, Franke MA, Michelsen PB, Bopp LH: Levofloxacin penetrates human monocytes and enhances intracellular killing of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Antimicrob Chemother 45:483-488, 2000.

272. Desplaces N, Acar JF: New quinolones in the treatment of joint and bone infections. Rev Infect Dis 10 Suppl 1:S179-183, 1988.

273. Darley ES, MacGowan AP: Antibiotic treatment of gram-positive bone and joint infections. J Antimicrob Chemother 53:928-935, 2004.

274. Blondeau JM, Laskowski R, Bjarnason J, Stewart C: Comparative in vitro activity of gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin and trovafloxacin against 4151 Gram-negative and Gram-positive organisms. Int J Antimicrob Agents 14:45-50, 2000.

275. Kaatz GW, Seo SM, Barriere SL, Albrecht LM, Rybak MJ: Efficacy of fleroxacin in experimental methicillin-resistant *Staphylococcus aureus* endocarditis. Antimicrob Agents Chemother 33:519-521, 1989.

276. Carpenter TC, Hackbarth CJ, Chambers HF, Sande MA: Efficacy of ciprofloxacin for experimental endocarditis caused by methicillin-susceptible or -resistant strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 30:382-384, 1986.

277. Chuard C, Herrmann M, Vaudaux P, Waldvogel FA, Lew DP: Successful therapy of experimental chronic foreign-body infection due to methicillin-resistant *Staphylococcus aureus* by antimicrobial combinations. Antimicrob Agents Chemother 35:2611-2616, 1991.

278. Dworkin RJ, Lee BL, Sande MA, Chambers HF: Treatment of right-sided *Staphylococcus aureus* endocarditis in intravenous drug users with ciprofloxacin and rifampicin. Lancet 2:1071-1073, 1989.

279. Heldman AW, Hartert TV, Ray SC, Daoud EG, Kowalski TE, Pompili VJ, Sisson SD, Tidmore WC, vom Eigen KA, Goodman SN, Lietman PS, Petty BG, Flexner C: Oral antibiotic treatment of right-sided staphylococcal endocarditis in injection drug users: prospective randomized comparison with parenteral therapy. Am J Med 101:68-76, 1996.

280. Schrenzel J, Harbarth S, Schockmel G, Genne D, Bregenzer T, Flueckiger U, Petignat C, Jacobs F, Francioli P, Zimmerli W, Lew D: A randomized clinical trial to compare fleroxacin-rifampicin with flucloxacillin or vancomycin for the treatment of staphylococcal infection. Clin Infect Dis 39:1285-292, 2004.

281. Drancourt M, Stein A, Argenson JN, Roiron R, Groulier P, Raoult D: Oral treatment of Staphylococcus spp. infected orthopaedic implants with fusidic acid or ofloxacin in combination with rifampicin. J Antimicrob Chemother 39:235-240, 1997.

282. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE: Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 279:1537-1541, 1998.

283. Sierra JM, Marco F, Ruiz J, Jimenez de Anta MT, Vila J: Correlation between the activity of different fluoroquinolones and the presence of mechanisms of quinolone resistance in epidemiologically related and unrelated strains of methicillin-susceptible and -resistant *Staphylococcus aureus*. Clin Microbiol Infect 8:781-790, 2002.

284. Hooper DC: Fluoroquinolone resistance among gram-positive cocci. Lancet Infect Dis 2:530-538, 2002.

285. Coronado VG, Edwards JR, Culver DH, Gaynes RP: Ciprofloxacin resistance among nosocomial *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the United States. National Nosocomial Infections Surveillance (NNIS) System. Infect Control Hosp Epidemiol 16:71-75, 1995.

286. Kresken M, Hafner D, Mittermayer H, Verbist L, Bergogne-Berezin E, Giamarellou H, Esposito S, van Klingeren B, Kayser FH, Reeves DS, et al.: Prevalence of fluoroquinolone resistance in Europe. Study Group 'Bacterial Resistance' of the Paul-Ehrlich-Society for chemotherapy e. V. Infection 22 Suppl 2:S90-98, 1994.

287. Weber SG, Gold HS, Hooper DC, Karchmer AW, Carmeli Y: Fluoroquinolones and the risk for methicillin-resistant *Staphylococcus aureus* in hospitalized patients. Emerg Infect Dis 9:1415-1422, 2003.

288. Thornsberry C: Susceptibility of clinical bacterial isolates to ciprofloxacin in the United States. Infection 22 Suppl 2:S80-89, 1994.

289. Cunha BA, Qadri SM, Ueno Y, Walters EA, Domenico P: Antibacterial activity of trovafloxacin against nosocomial Gram-positive and Gram-negative isolates. J Antimicrob Chemother 39 Suppl B:29-34, 1997.

290. Harbarth S, Liassine N, Dharan S, Herrault P, Auckenthaler R, Pittet D: Risk factors for persistent carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 31:1380-1385, 2000.

291. Charbonneau P, Parienti JJ, Thibon P, Ramakers M, Daubin C, du Cheyron D, Lebouvier G, Le Coutour X, Leclercq R: Fluoroquinolone use and methicillin-resistant *Staphylococcus aureus* isolation rates in hospitalized patients: a quasi experimental study. Clin Infect Dis 42:778-784, 2006.

292. Dalhoff A, Schmitz FJ: In vitro antibacterial activity and pharmacodynamics of new quinolones. Eur J Clin Microbiol Infect Dis 22:203-221, 2003.

293. Kumana CR, Yuen KY: Parenteral aminoglycoside therapy. Selection, administration and monitoring. Drugs 47:902-913, 1994.

294. Lucet JC, Herrmann M, Rohner P, Auckenthaler R, Waldvogel FA, Lew DP: Treatment of experimental foreign body infection caused by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 34:2312-2317, 1990.

295. Wilson WR, Karchmer AW, Dajani AS, Taubert KA, Bayer A, Kaye D, Bisno AL, Ferrieri P, Shulman ST, Durack DT: Antibiotic treatment of adults with infective endocarditis due to

streptococci, enterococci, staphylococci, and HACEK microorganisms. American Heart Association. Jama 274:1706-1713, 1995.

296.   Francioli PB, Freedman LR: Streptococcal infection of endocardial and other intravascular vegetations in rabbits: natural history and effect of dexamethasone. Infect Immun 24:483-491, 1979.

297.   Chambers HF, Miller RT, Newman MD: Right-sided *Staphylococcus aureus* endocarditis in intravenous drug abusers: two-week combination therapy. Ann Intern Med 109:619-624, 1988.

298.   DiNubile MJ: Short-course antibiotic therapy for right-sided endocarditis caused by *Staphylococcus aureus* in injection drug users. Ann Intern Med 121:873-876, 1994.

299.   Wallace JR, Goldstein E: Management of *Staphylococcus aureus* bacteremia. Compr Ther 20:437-440, 1994.

300.   Fortun J, Perez-Molina JA, Anon MT, Martinez-Beltran J, Loza E, Guerrero A: Right-sided endocarditis caused by *Staphylococcus aureus* in drug abusers. Antimicrob Agents Chemother 39:525-528, 1995.

301.   Fortun J, Navas E, Martinez-Beltran J, Perez-Molina J, Martin-Davila P, Guerrero A, Moreno S: Short-course therapy for right-side endocarditis due to *Staphylococcus aureus* in drug abusers: cloxacillin versus glycopeptides in combination with gentamicin. Clin Infect Dis 33:120-125, 2001.

302.   Moss R, Munt B: Injection drug use and right sided endocarditis. Heart 89:577-581, 2003.

303.   Zimmerli W, Trampuz A, Ochsner PE: Prosthetic-joint infections. N Engl J Med 351:1645-1654, 2004.

304.   Widmer AF, Pittet D: Optimal duration of therapy for catheter-related *Staphylococcus aureus* bacteremia. Clin Infect Dis 14:1259-1260, 1992.

305.   Bamberger DM: Diagnosis and treatment of osteomyelitis. Compr Ther 26:89-95, 2000.

306.   Drancourt M, Stein A, Argenson JN, Zannier A, Curvale G, Raoult D: Oral rifampin plus ofloxacin for treatment of Staphylococcus-infected orthopedic implants. Antimicrob Agents Chemother 37:1214-1218, 1993.

307.   Black J, Hunt TL, Godley PJ, Matthew E: Oral antimicrobial therapy for adults with osteomyelitis or septic arthritis. J Infect Dis 155:968-972, 1987.

308.   Langlais F: Can we improve the results of revision arthroplasty for infected total hip replacement? J Bone Joint Surg Br 85:637-640, 2003.

309.   Lew DP, Waldvogel FA: Osteomyelitis. Lancet 364:369-379, 2004.

310.   Bishara J, Leibovici L, Gartman-Israel D, Sagie A, Kazakov A, Miroshnik E, Ashkenazi S, Pitlik S: Long-term outcome of infective endocarditis: the impact of early surgical intervention. Clin Infect Dis 33:1636-1643, 2001.

311. Yu VL, Fang GD, Keys TF, Harris AA, Gentry LO, Fuchs PC, Wagener MM, Wong ES: Prosthetic valve endocarditis: superiority of surgical valve replacement versus medical therapy only. Ann Thorac Surg 58:1073-1077, 1994.

312. Roder BL, Wandall DA, Espersen F, Frimodt-Moller N, Skinhoj P, Rosdahl VT: A study of 47 bacteremic *Staphylococcus aureus* endocarditis cases: 23 with native valves treated surgically and 24 with prosthetic valves. Scand Cardiovasc J 31:305-309, 1997.

313. Mathew J, Abreo G, Namburi K, Narra L, Franklin C: Results of surgical treatment for infective endocarditis in intravenous drug users. Chest 108:73-77, 1995.

314. Arbulu A, Asfaw I: Tricuspid valvulectomy without prosthetic replacement. Ten years of clinical experience. J Thorac Cardiovasc Surg 82:684-691, 1981.

315. Straumann E, Stulz P, Jenzer HR: Tricuspid valve endocarditis in the drug addict: a reconstructive approach ("vegetectomy"). Thorac Cardiovasc Surg 38:291-294, 1990.

316. Fournier B, Philpott DJ: Recognition of *Staphylococcus aureus* by the innate immune system. Clin Microbiol Rev 18:521-540, 2005.

317. Sriskandan S, Cohen J: Gram-positive sepsis. Mechanisms and differences from gram-negative sepsis. Infect Dis Clin North Am 13:397-412, 1999.

318. Kaplan JE, Palmer DL, Tung KS: Teichoic acid antibody and circulating immune complexes in the management of *Staphylococcus aureus* bacteremia. Am J Med 70:769-774, 1981.

319. Tarkowski A: Pathogenetic events in experimental *Staphylococcus aureus* infections. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 102-119.

320. Verbrugh HA, Peters R, Goessens WH, Michel MF: Distinguishing complicated from uncomplicated bacteremia caused by *Staphylococcus aureus*: the value of "new" and "old" serological tests. J Infect Dis 153:109-115, 1986.

321. Dziarski R: Comparison of in vitro and in vivo mitogenic and polyclonal antibody and autoantibody responses to peptidoglycan, LPS, protein A, PWM, PHA and Con A in normal and autoimmune mice. J Clin Lab Immunol 16:93-109, 1985.

322. Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, Nicholson G, Neumeister B, Mond JJ, Peschel A: Role of teichoic acids *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med 10:243-245, 2004.

323. Hofstad T: The genus staphylococcus. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 14-32.

324. Patti JM, Hook M: Microbial adhesins recognizing extracellular matrix macromolecules. Curr Opin Cell Biol 6:752-758, 1994.

325. Ryden C, Yacoub AI, Maxe I, Heinegard D, Oldberg A, Franzen A, Ljungh A, Rubin K: Specific binding of bone sialoprotein to *Staphylococcus aureus* isolated from patients with osteomyelitis. Eur J Biochem 184:331-336, 1989.

326. Patti JM, Allen BL, McGavin MJ, Hook M: MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585-617, 1994.

327. Balwit JM, van Langevelde P, Vann JM, Proctor RA: Gentamicin-resistant menadione and hemin auxotrophic *Staphylococcus aureus* persist within cultured endothelial cells. J Infect Dis 170:1033-1037, 1994.

328. Sinha B, Francois PP, Nusse O, Foti M, Hartford OM, Vaudaux P, Foster TJ, Lew DP, Herrmann M, Krause KH: Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. Cell Microbiol 1:101-117, 1999.

329. Solberg CO: Host defence in staphylococcal infections. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 148-163.

330. Bokarewa MI, Jin T, Tarkowski A: *Staphylococcus aureus*: staphylokinase. Int J Biochem Cell Biol 38:504-509, 2006.

331. Christner RB, Boyle MD: Role of staphylokinase in the acquisition of plasmin(ogen)-dependent enzymatic activity by staphylococci. J Infect Dis 173:104-112, 1996.

332. Molkanen T, Tyynela J, Helin J, Kalkkinen N, Kuusela P: Enhanced activation of bound plasminogen on *Staphylococcus aureus* by staphylokinase. FEBS Lett 517:72-78, 2002.

333. Jin T, Bokarewa M, McIntyre L, Tarkowski A, Corey GR, Reller LB, Fowler VG, Jr.: Fatal outcome of bacteraemic patients caused by infection with staphylokinase-deficient *Staphylococcus aureus* strains. J Med Microbiol 52:919-923, 2003.

334. Haslinger-Loffler B, Kahl BC, Grundmeier M, Strangfeld K, Wagner B, Fischer U, Cheung AL, Peters G, Schulze-Osthoff K, Sinha B: Multiple virulence factors are required for *Staphylococcus aureus* -induced apoptosis in endothelial cells. Cell Microbiol 7:1087-1097, 2005.

335. Cribier B, Prevost G, Couppie P, Finck-Barbancon V, Grosshans E, Piemont Y: *Staphylococcus aureus* leukocidin: a new virulence factor in cutaneous infections? An epidemiological and experimental study. Dermatology 185:175-180, 1992.

336. Colin DA, Mazurier I, Sire S, Finck-Barbancon V: Interaction of the two components of leukocidin from *Staphylococcus aureus* with human polymorphonuclear leukocyte membranes: sequential binding and subsequent activation. Infect Immun 62:3184-3188, 1994.

337. Konig B, Koller M, Prevost G, Piemont Y, Alouf JE, Schreiner A, Konig W: Activation of human effector cells by different bacterial toxins (leukocidin, alveolysin, and erythrogenic toxin A): generation of interleukin-8. Infect Immun 62:4831-4837, 1994.

338. Zetola N, Francis JS, Nuermberger EL, Bishai WR: Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis 5:275-286, 2005.

339. Yamasaki O, Kaneko J, Morizane S, Akiyama H, Arata J, Narita S, Chiba J, Kamio Y, Iwatsuki K: The Association between *Staphylococcus aureus* strains carrying panton-valentine leukocidin genes and the development of deep-seated follicular infection. Clin Infect Dis 40:381-385, 2005.

340. Etienne J: Panton-Valentine leukocidin: a marker of severity for *Staphylococcus aureus* infection? Clin Infect Dis 41:591-593, 2005.

341. Balaban N, Rasooly A: Staphylococcal enterotoxins. Int J Food Microbiol 61:1-10, 2000.

342. McCormick JK, Yarwood JM, Schlievert PM: Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol 55:77-104, 2001.

343. Bremell T, Tarkowski A: Preferential induction of septic arthritis and mortality by superantigen-producing staphylococci. Infect Immun 63:4185-4187, 1995.

344. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233-2239, 1995.

345. Rosdahl VT: Epidemiology. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 78-98.

346. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG: Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38:1008-1015, 2000.

347. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K: Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist Updat 6:41-52, 2003.

348. Oliveira DC, Tomasz A, de Lencastre H: Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. Lancet Infect Dis 2:180-189, 2002.

349. Saravolatz LD, Markowitz N, Arking L, Pohlod D, Fisher E: Methicillin-resistant *Staphylococcus aureus*. Epidemiologic observations during a community-acquired outbreak. Ann Intern Med 96:11-16, 1982.

350. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B: Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 352:1445-1453, 2005.

351. Kerttula AM, Lyytikainen O, Salmenlinna S, Vuopio-Varkila J: Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Finland. J Hosp Infect 58:109-114, 2004.

352.   Kerttula AM, Lyytikäinen O, Virolainen-Julkunen A, Vuopio-Varkila J: Perimätutkimukset valaisevat Suomen huonontuneen MRSA-tilanteen taustaa. Suomen Lääkärilehti 21-22:2383-2387, 2006.

353.   Salmenlinna S, Lyytikainen O, Vuopio-Varkila J: Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. Emerg Infect Dis 8:602-607, 2002.

354.   Shukla SK: Community-associated methicillin-resistant *Staphylococcus aureus* and its emerging virulence. Clin Med Res 3:57-60, 2005.

355.   Deresinski S: Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. Clin Infect Dis 40:562-573, 2005.

356.   Larinkari UM, Valtonen MV, Sarvas M, Valtonen VV: Teichoic acid antibody test: its use in patients with coagulase-positive staphylococcal bacteremia. Arch Intern Med 137:1522-1525, 1977.

357.   Crowder JG, White A: Teichoic acid antibodies in staphylococcal and nonstaphylococcal endocarditis. Ann Intern Med 77:87-90, 1972.

358.   Bayer AS: Staphylococcal bacteremia and endocarditis: state of the art. Arch Intern Med 142:1169-1177, 1982.

359.   Tuazon CU, Sheagren JN, Choa MS, Marcus D, Curtin JA: *Staphylococcus aureus* bacteremia: relationship between formation of antibodies to teichoic acid and development of metastatic abscesses. J Infect Dis 137:57-62, 1978.

360.   Christensson B: Serological and other non-culture diagnostic assays. In: Espersen F, Heström SÅ, Solberg CO, eds. The Ever Present Pathogens: An update on staphylococci and staphylococcal infections. The Authors and Pharmacia & Upjohn: Rosell & Co, 1999: 166-183.

361.   Nagel JG, Tuazon CU, Cardella TA, Sheagren JN: Teichoic acid serologic diagnosis of staphylococcal endocarditis. Use of gel diffusion and counterimmunoelectrophoretic methods. Ann Intern Med 82:13-17, 1975.

362.   Tuazon CU, Sheagren JN: Teichoic acid antibodies in the diagnosis of serious infections with *Staphylococcus aureus*. Ann Intern Med 84:543-546, 1976.

363.   White A, Wheat LJ, Kohler RB: Diagnostic and therapeutic significance of staphylococcal teichoic acid antibodies. Scand J Infect Dis Suppl 41:105-116, 1983.

364.   Granstrom M, Julander I, Mollby R: Serological diagnosis of deep *Staphylococcus aureus* infections by enzyme-linked immunosorbent assay (ELISA) for staphylococcal hemolysins and teichoic acid. Scand J Infect Dis Suppl 41:132-139, 1983.

365.   Ryding U, Espersen F, Soderquist B, Christensson B: Evaluation of seven different enzyme-linked immunosorbent assays for serodiagnosis of *Staphylococcus aureus* bacteremia. Diagn Microbiol Infect Dis 42:9-15, 2002.

366.   Tenenbaum MJ, Archer GL: Prognostic value of teichoic acid antibodies in *Staphylococcus aureus* bacteremia: a reassessment. South Med J 73:140-143, 149, 1980.

367. Bayer AS, Tillman DB, Concepcion N, Guze LB: Clinical value of teichoic acid antibody titers in the diagnosis and management of the staphylococcemias. West J Med 132:294-300, 1980.

368. Thompson RL: Staphylococcal infective endocarditis. Mayo Clin Proc 57:106-114, 1982.

369. Wise KA, Tosolini FA: Detection of teichoic acid antibodies in *Staphylococcus aureus* infections. Pathology 24:102-108, 1992.

370. Larinkari U: Assay of teichoic acid antibodies and antistaphylolysin in the diagnosis of Staphylococcal osteomyelitis. Scand J Infect Dis 14:123-126, 1982.

371. Larinkari U, Valtonen VV: Comparison of anti-alpha-hemolysin and teichoic acid antibody tests in patients with endocarditis and septicaemia caused by *Staphylococcus aureus*. Scand J Infect Dis Suppl 41:144-147, 1983.

372. Larinkari U, Valtonen VV: Comparison of anti-alpha-haemolysin and teichoic acid antibody tests in patients with *Staphylococcus aureus* endocarditis or bacteraemia. J Infect 8:221-226, 1984.

373. Larinkari U: Serum antibody to staphylococcal teichoic acid and alpha-haemolysin in dermatological patients. Br J Dermatol 107:53-58, 1982.

374. Mustakallio KK: Antistaphylolysin (ASta) level of the blood in relation to barrier function of the skin. Multivariate analysis of survey data of 593 hospitalized patients with Besnier's prurigo (atopic eczema). Ann Med Exp Biol Fenn 44:Suppl 7:1-53, 1966.

375. Espersen F, Hedstrom SA: Precipitating antibodies against *Staphylococcus aureus* in serum from patients with staphylococcal septicaemia, investigated by means of quantitative immunoelectrophoretic methods. Acta Pathol Microbiol Scand [B] 89:261-266, 1981.

376. Colque-Navarro P, Soderquist B, Holmberg H, Blomqvist L, Olcen P, Mollby R: Antibody response in *Staphylococcus aureus* septicaemia - a prospective study. J Med Microbiol 47:217-225, 1998.

377. McCabe WR, Jackson GG: Gram negative bacteraemia 1. Etiology and ecology. Arch Intern Med 110:847-855, 1962.

378. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 31:1250-1256, 2003.

379. Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE, Fussing V, Salmenlinna S, Vuopio-Varkila J, El Solh N, Cuny C, Witte W, Tassios PT, Legakis N, van Leeuwen W, van Belkum A, Vindel A, Laconcha I, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Coombes G, Cookson B: Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41:1574-1585, 2003.

380. Goering RV, Winters MA: Rapid method for epidemiological evaluation of gram-positive cocci by field inversion gel electrophoresis. J Clin Microbiol 30:577-580, 1992.

381. Brakstad OG, Aasbakk K, Maeland JA: Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol 30:1654-1660, 1992.

382. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, Reller LB: The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 24:584-602, 1997.

383. Diekema DJ, Pfaller MA, Jones RN, Doern GV, Kugler KC, Beach ML, Sader HS: Trends in antimicrobial susceptibility of bacterial pathogens isolated from patients with bloodstream infections in the USA, Canada and Latin America. SENTRY Participants Group. Int J Antimicrob Agents 13:257-271, 2000.

384. Fluit AC, Jones ME, Schmitz FJ, Acar J, Gupta R, Verhoef J: Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. Clin Infect Dis 30:454-460, 2000.

385. Siegman-Igra Y, Fourer B, Orni-Wasserlauf R, Golan Y, Noy A, Schwartz D, Giladi M: Reappraisal of community-acquired bacteremia: a proposal of a new classification for the spectrum of acquisition of bacteremia. Clin Infect Dis 34:1431-439, 2002.

386. Smith GE, Cook DA: *Streptococcus pyogenes* and *Staphylococcus aureus* pseudo-bacteremia. J Infect 18:194-195, 1989.

387. Julander I, Arneborn P, Back E, Hoglund C, Svanbom M: Intravenous drug addiction - staphylococcal septicemia - pulmonary embolism: a triad pathognomonic for tricuspid valve endocarditis? Scand J Infect Dis 15:257-265, 1983.

388. Murphy EL, DeVita D, Liu H, Vittinghoff E, Leung P, Ciccarone DH, Edlin BR: Risk factors for skin and soft-tissue abscesses among injection drug users: a case-control study. Clin Infect Dis 33:35-40, 2001.

389. Torres-Tortosa M, de Cueto M, Vergara A, Sanchez-Porto A, Perez-Guzman E, Gonzalez-Serrano M, Canueto J: Prospective evaluation of a two-week course of intravenous antibiotics in intravenous drug addicts with infective endocarditis. Grupo de Estudio de Enfermedades Infecciosas de la Provincia de Cadiz. Eur J Clin Microbiol Infect Dis 13:559-564, 1994.

390. Perlman BB, Freedman LR: Experimental endocarditis. 3. Natural history of catheter induced staphylococcal endocarditis following catheter removal. Yale J Biol Med 44:214-224, 1971.

391. Sande MA, Johnson ML: Antimicrobial therapy of experimental endocarditis caused by *Staphylococcus aureus*. J Infect Dis 131:367-375, 1975.

392. Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, Etienne J, Richet H: Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis 35:819-824, 2002.