

# i. Gram-Positive Cocci

194

## *Staphylococcus aureus* (Including Staphylococcal Toxic Shock Syndrome)

Yok-Ai Que and Philippe Moreillon

### SHORT VIEW SUMMARY

#### Definition

- *Staphylococcus aureus* is a gram-positive pathogen that is responsible for superficial and deep-seated infections.
- It is a frequent colonizer of asymptomatic carriers.
- The organism is responsible for both pyogenic and toxin-related diseases.
- It is the primary cause of community- and hospital-acquired bloodstream infections and the first cause of invasive infections including infective endocarditis and osteomyelitis.
- Frequently, *S. aureus* is resistant to methicillin (methicillin-resistant *S. aureus* [MRSA]) and almost all β-lactam drugs (in up to 50% of hospital isolates).
- Often it is coresistant to many clinically available antibiotics.

#### Epidemiology

- *S. aureus* is a colonizer of the anterior nostrils in 20% to 40% of the normal population.
- With regard to drug-resistant nosocomial infections, it is the number one public health problem.
- Clonal spread of MRSA occurs from health care settings (health care-associated [HCA] MRSA) and other permissive environments (see Fig. 194.2).
- Polyclonal dispersion of susceptible strains occurs in the community, but successful clones

of community-acquired (CA) MRSA may spread worldwide (e.g., USA300).

- The organism is a superantigen producer responsible for toxic shock syndrome and food poisoning.

#### Microbiology

- *S. aureus* is the most virulent species of the more than 40 *Staphylococcus* spp. taxa.
- Its conserved core genome consists of approximately 2.8 million base pairs.
- It has multiple mobile genetic elements (MGEs): pathogenic and genomic islands, transposons, and prophages encoding virulence and antibiotic-resistance genes (see Tables 194.1 and 194.3).
- Multiple immune-evasion strategies are used by the organism, which impedes vaccine development (see Table 194.4).
- Evolution of successful clones occurs by means of mutations and acquisition of MGEs.
- Methicillin resistance is conferred by a polymorph family of SCCmec cassettes.

#### Diagnosis

- Conventional cultures are mandatory and critical for the detection of new resistance phenotypes.
- Molecular tests are useful for rapid identification of known drug-resistance genes.

- Molecular typing is critical for management of MRSA epidemics and infection control.

#### Therapy (See Tables 194.9 and 194.10)

- The first choice for methicillin-susceptible *S. aureus* is a penicillinase-resistant β-lactam or first-generation cephalosporin—for instance, cephalexin or dicloxacillin orally; nafcillin or oxacillin intravenously; or, outside the United States, flucloxacillin. Alternatives include clindamycin or trimethoprim-sulfamethoxazole (TMP-SMX) orally or intravenously.
- The first choice for MRSA would be vancomycin or daptomycin intravenously, or, if susceptible, TMP-SMX or clindamycin given orally or intravenously. Alternatives include ceftaroline, linezolid, or telavancin or, for acute bacterial skin and skin structure infections, dalbavancin, oritavancin, or tedizolid.
- A partner drug for combinations is rifampin.
- The benefit of aminoglycosides not well demonstrated and no longer recommended for native valve endocarditis.

#### Prevention

- Prevention measures include the following:
  - Decolonization of staphylococcal carriers (see Table 194.8)
  - Detection of HCA-MRSA and epidemic CA-MRSA
  - Hand hygiene measures
  - Vaccines in development

*Staphylococcus aureus* is a highly successful opportunistic pathogen. It is a frequent colonizer of the skin and mucosa of humans and animals (it is present in the anterior nares of up to 30% of the healthy human population) and can produce a wide variety of diseases. These diseases encompass relatively benign skin infections, such as folliculitis and furunculosis, and life-threatening conditions, including erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis.<sup>1</sup> In addition to infections in which the organism is physically present at the infected site, *S. aureus* is also capable of producing "distant" diseases, which are mediated by the secretion of toxins.<sup>2</sup> The toxins can be produced directly by bacteria that colonize the skin or mucosa or indirectly by microorganisms that colonize food or beverages. The former is exemplified by staphylococcal scalded skin syndrome (SSSS),<sup>3,4</sup> which is the result of skin, mucosal, or wound colonization by *S. aureus*-producing exfoliative toxin A or B (ETA or ETB) and by staphylococcal

toxic shock syndrome (TSS),<sup>2,5</sup> which is the result of the production of toxic shock syndrome toxin 1 (TSST-1) or exotoxins B or C. The latter is exemplified by *S. aureus* food intoxication, in which the toxin is ingested with the contaminated dish, and disease follows shortly thereafter in the form of vomiting and diarrhea. Food intoxication is the result of staphylococcal toxins called enterotoxins.<sup>2,6</sup> These toxins are heat stable. Cooking may kill the contaminants but does not denature the toxins. Hence, subsequent culture of the dish may fail to grow the culprit bacterium.

*S. aureus* has an extraordinary capacity to adapt and survive in a great variety of environments. During the past decades, molecular and genetic dissection of *S. aureus* has revealed a great number of surface adhesins, which mediate adherence to and colonization of target tissues, and secreted enzymes, toxins, superantigens (SAGs) and immune evasion determinants that are responsible for invasion and distant disease

---

Revised July 1, 2020

(Table 194.1).<sup>1,7–9</sup> The availability of now several thousands of *S. aureus* genome assemblies and annotation reports ([www.ncbi.nlm.nih.gov/genome/genomes/154](http://www.ncbi.nlm.nih.gov/genome/genomes/154)) has helped complete this portrait. *S. aureus* is part of the Firmicutes phylum and shares approximately 50% of orthologue genes with notoriously nonpathogenic *Bacillus subtilis*, which indicates that the two organisms have evolved from a common ancestor.<sup>10–12</sup> Homology searches on the chromosome revealed numerous new surface-attached and secreted factors that represent additional pathogenic factors. *S. aureus* harbors a large number of mobile genetic elements (MGEs) from exogenous origin, including insertion sequences, transposons, bacteriophages, pathogenicity islands, and genomic islands, which contain specific determinants responsible for disease and antibiotic resistance.<sup>7,8,10,13,14</sup> The presence of these exogenous elements attests to the high capacity of *S. aureus* to undergo horizontal gene transfer and exchange genetic elements with other organisms, including staphylococcal and nonstaphylococcal genera. Because gene exchange is a key player of evolution, this peculiar genetic plasticity is a likely explanation for the success of *S. aureus*, both as a colonizer and a disease-producing microbe. In the case of SAGs (see later discussion), one of the trading partner is suspected to be *Streptococcus pyogenes*.<sup>2</sup>

### THE MICROORGANISM

Members of the *Staphylococcus* genus are gram-positive cocci (0.5–1.5 μm in diameter) that occur singly and in pairs, tetrads, short chains, and irregular grapelike clusters. Ogston<sup>15</sup> introduced the name *Staphylococcus* (Greek *staphylé*, “a bunch of grapes”) to describe micrococci responsible for inflammation and suppuration. Staphylococci are nonmotile, non-spore forming, and usually catalase positive, and they are often unencapsulated or have a limited capsule (Fig. 194.1). Most species are facultative anaerobes.<sup>11,12</sup>

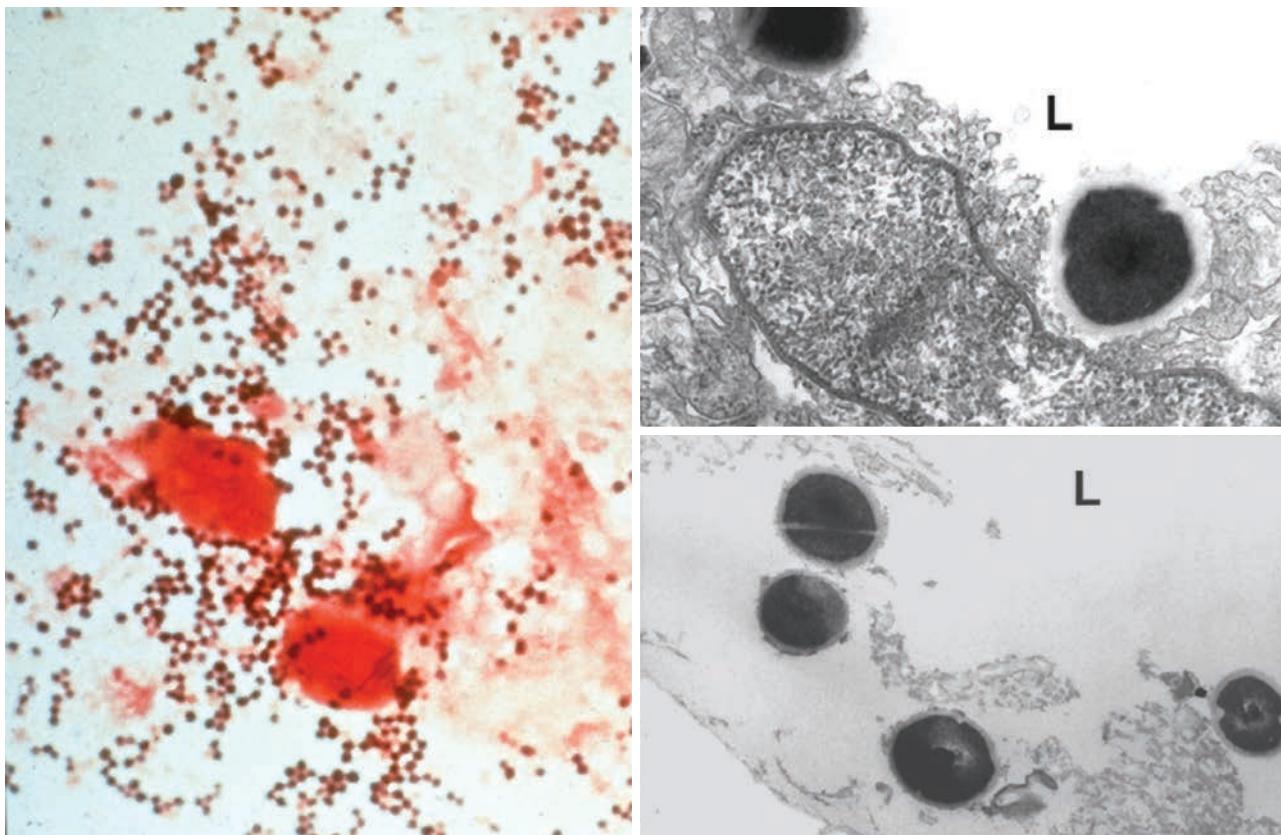
The genus *Staphylococcus* contains up to 40 taxa, 16 of which are commonly found in humans (Table 194.2). Only a few are pathogenic in the absence of predisposing immunosuppression or implanted foreign material. The most virulent ones include *S. aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi* in humans, and *S. aureus* and *Staphylococcus intermedius* in animals. Although *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus saprophyticus* are commonly responsible for device-related and urinary tract infections, they produce substantially less devastating disease syndromes than *S. aureus*.

*S. aureus* harbors some unique features when compared with its less-disease-producing congeners. These include coagulase and clumping factors (or fibrinogen-binding proteins), which have laboratory diagnostic value because they help rapidly discriminate between coagulase-positive (i.e., *S. aureus*) and coagulase-negative staphylococci (CoNS; see Table 194.2). Moreover, *S. aureus* carries between more than 20 and more than 30 adhesin and toxin genes, respectively, as compared with 10 or fewer adhesin genes and virtually no toxin genes for the CoNS mentioned previously.<sup>7,16–18</sup> Thus, *S. aureus* is a distinct pathogen within the *Staphylococcus* genus.

### Habitat

Staphylococci are ubiquitous colonizers of the skin and mucosa of virtually all animals, including mammals and birds.<sup>11,12</sup> Some species have preferential niches as indicated by their names (see Table 194.2). *S. epidermidis* and *Staphylococcus capitis* are constant colonizers of the skin and scalp, respectively. *Staphylococcus pseudintermedius* is a colonizer of cats and dogs and may be misidentified as *S. aureus* (tube coagulase positive, slide coagulase negative) when transmitted by animal bites.<sup>19</sup>

In animals, *S. aureus* is a major cause of livestock infection, including mastitis in bovine and ovine herds. In humans, *S. aureus* has a niche



**FIG. 194.1** Gram staining and transmission electron microscopy of clinical samples of *Staphylococcus aureus*. Left, Gram-stained sputum specimen from 20-year-old patient with fulminant hemorrhagic *S. aureus* pneumonia (see also Fig. 194.8). Grapelike clusters of bacteria and eukaryotic cells are visible. Right, Electron microscopy images from rat with experimental aortic endocarditis caused by *S. aureus*. Upper part depicts *S. aureus* in process of invading valve endothelial cell. Lower part depicts endothelial cell that has been lysed by invading bacteria, probably as a result of *S. aureus* hemolysin secretion. L, Lumen side of endothelium.

**TABLE 194.1 Some of the Major *Staphylococcus aureus* Extracellular Factors Involved in Pathogenesis and Response to Global Regulatory Elements During Bacterial Growth**

GENE	LOCATION	PRODUCT	ACTIVITY OR FUNCTION	TIMING <sup>a</sup>	ACTION OF REGULATORY GENES <sup>b</sup>			
					agr	saeRS	rot	sarA
<b>Surface Proteins</b>								
spa	Chromosome	Protein A	Blocks IgGs, binds von Willebrand factor	Exp	–	See footnote <sup>c</sup>	+	
cna	Chromosome	Collagen BP	Collagen binding	Exp	–			
fnbA	Chromosome	Fibronectin BPA	Fibronectin binding	Exp	–		–	
fnbB	Chromosome	Fibronectin BBP	Fibronectin binding	Exp	–		+	
clfA	Chromosome	Clumping factor A	Fibrinogen binding	Exp	0			
clfB	Chromosome	Clumping factor B	Fibrinogen binding	Exp	0	+	0	
sdrC	Chromosome	Serine-aspartate repeat protein	Fibrinogen binding Cytokeratin binding	Exp				+
<b>Capsular Polysaccharides</b>								
cap5	Chromosome	Polysaccharide capsule type 5	Antiphagocytosis	Pxp	+			+
cap8	Chromosome	Polysaccharide capsule type 8	Antiphagocytosis	Pxp	+			
<b>Cytotoxins</b>								
hla	Chromosome	α-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote <sup>c</sup>
hlb	Chromosome	β-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote <sup>c</sup>
hld	Chromosome	δ-Hemolysin	Hemolysin, cytotoxin	Pxp	+	0		+
hlg	Chromosome	γ-Hemolysin	Hemolysin, cytotoxin	Pxp	+		–	See footnote <sup>c</sup>
lukS/F	PVL phage	PVL	Leucocidin	Pxp	+		–	
<b>Superantigens</b>								
sea	Bacteriophage	Enterotoxin A	Food poisoning, TSS	Xp	0			
seb	SaPI3 <sup>d</sup>	Enterotoxin B	Food poisoning, TSS	Pxp	+			See footnote <sup>c</sup>
sec	SaPI4 <sup>d</sup>	Enterotoxin C	Food poisoning, TSS	Pxp	+			
sed	Plasmid	Enterotoxin D	Food poisoning, TSS	Pxp	+			
eta	ETA phage	Exfoliatin A	Scalded skin syndrome	Pxp	+			
etb	Plasmid	Exfoliatin B	Scalded skin syndrome	Pxp	+			
tst	SaPI1,2, bov1 <sup>d</sup>	Toxic shock toxin 1	TSS	Pxp	+			See footnote <sup>c</sup>
<b>Enzymes</b>								
SpA-F	Chromosome	Serine protease-like	Putative protease		+		–	
ssp	Chromosome	V8 protease	Spreading factor	Pxp	+	0		–
aur	Chromosome	Metalloprotease (aureolysin)	Processing enzyme?	Pxp	+			–
sspB	Chromosome	Cysteine protease	Processing enzyme?	?			–	
scp	Chromosome	Staphopain (protease II)	Spreading, nutrition	Pxp	+			–
geh	Chromosome	Glycerol ester hydrolase	Spreading, nutrition	Pxp	+	0	–	See footnote <sup>c</sup>
lip	Chromosome	Lipase (butyryl esterase)	Spreading, nutrition	Pxp	+	0		See footnote <sup>c</sup>
fme	Chromosome	FAME	Fatty acid esterification	Pxp	+			See footnote <sup>c</sup>
plc	Chromosome	PI-phospholipase C		Pxp	+			
nuc	Chromosome	Nuclease	Nutrition	Pxp	+	+		
has	Chromosome	Hyaluronidase	Spreading factor	Xp	See footnote <sup>c</sup>			
coa	Chromosome	Coagulase	Clotting, clot digestion	Exp		+	+	+
sak	Bacteriophage	Staphylokinase	Plasminogen activator	Pxp	+	0		

<sup>a</sup>Timing: Xp, throughout exponential phase; Exp, early exponential phase only; Pxp, postexponential phase; 0, no effect of gene on. Expression: +, upregulated; –, downregulated.

<sup>b</sup>agr, Accessory gene regulator; PVL, Panton-Valentine leukocidin; saeRS, *S. aureus* exoproteins; rot, repressor of toxins; sarA, *Staphylococcus* accessory regulator.

<sup>c</sup>Controversial.

<sup>d</sup>SaPI, *S. aureus* pathogenic island.

BP, Binding protein; FAME, fatty acid modifying enzyme; TSS, toxic shock syndrome.

Modified from Cheung AL, Projan SJ, Gresham H. The genomic aspect of virulence, sepsis, and resistance to killing mechanisms in *Staphylococcus aureus*. *Curr Infect Dis Rep*. 2002;4:400–410; and Novick RP, Geisinger E. Quorum sensing in staphylococci. *Ann Rev Genet*. 2008;42:541–564.

**TABLE 194.2 Some Staphylococcal Species From Mammals and Relationship Between Production of Coagulase and Clumping Factor (Fibrinogen-Binding Protein A) and Potential Virulence**

HOST	SPECIES	COAGULASE <sup>a</sup>	CLUMPING FACTOR <sup>a</sup>	VIRULENCE <sup>a</sup>
Human and other primates	<i>S. aureus</i>	++	++	+++
	<i>S. epidermidis</i>	-	-	+
	<i>S. capitis</i>	-	-	±
	<i>S. caprae</i>	-	-	±
	<i>S. saccharolyticus</i>	±	-	-
	<i>S. warneri</i>	-	-	-
	<i>S. pasteuri</i>	-	-	-
	<i>S. haemolyticus</i>	-	-	+
	<i>S. hominis</i>	-	-	±
	<i>S. lugdunensis</i>	-	±	+
	<i>S. auricularis</i>	-	-	±
	<i>S. saprophyticus</i>	-	-	+
	<i>S. cohnii</i>	-	-	-
	<i>S. xilosus</i>	-	-	-
	<i>S. simulans</i>	-	-	-
	<i>S. schleiferi</i>	±	+	+
Carnivores	<i>S. intermedius</i>	+	-	++
	<i>S. felis</i>	-	-	++

<sup>a</sup>Semiquantitative estimate of production of coagulase and clumping factor and relation to virulence.

Modified from Kloos WE, Schleifer KH, Goetz F. The genus *Staphylococcus*. In: Balows A, Trüper HG, Dworkin M, et al, eds. *The Prokaryotes*. 2nd ed. New York: Springer-Verlag; 1992:1369–1420; and Kloos WE, Bannerman TL. *Staphylococcus and Micrococcus*. In: Murray PR, Baron EJ, Pfaffer MA, et al, eds. *Manual of Clinical Microbiology*. 6th ed. Washington, DC: ASM Press; 1995:282–298.

preference for the anterior nares, especially in adults,<sup>20–22</sup> and is shed onto healthy skin, including axilla and perineum. However, certain clones may have preferences for more hidden niches, as was shown in the case of a peculiar epidemic hospital methicillin-resistant *S. aureus* (MRSA) clone that colonized the groin and rectum.<sup>23</sup> *S. aureus* can exist as a resident or a transient member of the normal flora. Nasal carrier rate varies from 10% to 40% in both the community and the hospital environment. *S. aureus* carriage in various anatomic sites may put certain populations at an increased risk for infection, such as patients with recurring furunculosis and patients who are subject to medical procedures, including hemodialysis, peritoneal dialysis, and surgery (see later section “Carriage of *Staphylococcus aureus*”).<sup>24–26</sup>

*S. aureus* carriage has also become a way of persistence and spread of multiresistant staphylococci, especially MRSA.<sup>20,21,23,26</sup> Because MRSA can resist many of the antibiotics in common use, it has risen to the level of a public health threat in the hospital for 3 decades and in the community since the beginning of this century.<sup>25,27</sup>

### Culture and Identification

Live organisms obtained by means of culture are critical for phenotypic diagnosis and revealing emerging antibiotic resistant phenotypes from as yet unknown mechanisms. In addition, molecular diagnosis helps speed up the results, which take a few hours instead of 1 to 3 days with bacterial subculturing. Molecular methods also help detect the presence of nonculturable microbes, mostly when patients have taken antibiotics before sample collection.

Techniques for culture and identification of staphylococci have been described.<sup>11</sup> Specimens should be inoculated both on blood agar and into rich liquid media such as Mueller-Hinton broth. With *S. aureus*, abundant growth occurs normally within 18 to 24 hours. However, morphologic variants (see subsequent discussion) may require prolonged growth periods, and plates should be kept 2 to 3 days in order to detect them. Colonies should be Gram stained, subcultured, and tested for genus, species, and antibiotic susceptibility when appropriate. Phenotypic tests for species identification include coagulase tests and agglutination tests, which detect the presence of surface determinants, including clumping factor, protein A, and polysaccharides.<sup>28</sup> Phenotypic antibiotic susceptibility tests vary from agar-diffusion methods (e.g., Kirby-Bauer and Etests) to automated measurement of metabolic activity or growth rates. Macro broth or agar dilution methods are precise but are not routinely performed in the laboratory.<sup>29</sup>

Molecular specification may be necessary in case of unclear phenotype, such as, for instance, in the case of morphologic variants (see next section).

### Morphologic Variants

Prolonged incubation is particularly important for the detection of morphology variants such as *small colony variants* (SCVs). SCVs grow into tiny colonies that are difficult to distinguish and may be mistakenly disregarded as contaminants.<sup>30</sup> They are usually recovered from protracted, difficult-to-treat infections such as chronic osteomyelitis and infected orthopedic prostheses, and have also been described in patients with cystic fibrosis.<sup>31</sup>

The most classic types of SCVs are selected during aminoglycoside therapy and result from alterations in the respiratory chain. Such SCVs have a lower transmembrane potential, which impedes the intake of the drug.<sup>32</sup> Interesting to note, switching from normal colonies to SCVs occurs naturally in the absence of antibiotic at a high rate (about 10<sup>-6</sup>), and switching back from SCVs to normal colonies also occurs.<sup>33</sup> Hence, SCVs are proposed to result from an intrinsic capacity of the bacterium to survive in unfavorable conditions rather than fortuitous mutations.

SCVs are also selected by other antimicrobial agents, including triclosan.<sup>34</sup> They were recovered from the sputa of up to 25% of children with cystic fibrosis and were statistically significantly associated with previous trimethoprim-sulfamethoxazole (TMP-SMX) therapy.<sup>31</sup> Such SCVs carry mutations in the thymidylate synthase gene (*thyA*) and are dependent on exogenous thymidine to grow.<sup>35</sup> *S. aureus* synthesizes thymidine by using *thyA* plus tetrahydrofolate to convert uridine monophosphate into thymidine monophosphate. TMP inhibits the synthesis of tetrahydrofolate, thus making *thyA* useless. By mutating the *thyA* gene, *S. aureus* forces itself to rely on exogenous vital thymidine by importing it. This makes the bacterium resistant to TMP. Thymidine is available in DNA-rich lung secretions of patients with cystic fibrosis and in abscesses. However, the rate of thymidine import is limiting, which results in slow growth and SCV phenotype.

In spite of slow growth, SCVs are equally as or more infective than their fast-growing parents in experimental infections such as osteoarthritis<sup>36</sup> and endocarditis.<sup>37</sup> Moreover, SCVs are particularly prone to invade eukaryotic cells and persist in them,<sup>30,38</sup> which may explain their occurrence in latent infections. SCVs are cross-resistant to drug-induced killing by most antibiotics,<sup>32</sup> and their eradication necessitates prolonged antibiotic therapy including drug combinations with rifampin.

### MOLECULAR DIAGNOSIS

Molecular diagnosis plays an increasing role in rapid detection of microbial pathogens and identification of drug-resistance determinants. Techniques based on molecular probing have been reviewed.<sup>39</sup> One of

these techniques relies on fluorescent detection of 16S rRNA with a peptide nucleic acid probe (peptide nucleic acid fluorescence in situ hybridization [PNA-FISH]). Such a technique has been shown to be highly specific and to help in discriminating *S. aureus* from CoNS in blood culture within 4 hours.<sup>40</sup> In addition, its usefulness in clinical therapeutic decision making has also been demonstrated.<sup>41</sup>

Multiplex real-time polymerase chain reaction (PCR) assays are being developed to quantify organisms directly in clinical samples. Genes representative of both species and resistance mechanisms are amplified simultaneously. For MRSA, the resistance gene sought is *mecA*, which encodes low-affinity penicillin-binding protein A (PBP2A).

However, *mecA* is also present in methicillin-resistant CoNS and thus detects simultaneously both MRSA and commensal methicillin-resistant CoNS, which may result in false diagnosis. One way to bypass this limit is to extend the *mecA* amplification product to *orfX*. *orfX* is an open reading frame that is specific for *S. aureus*, and its amplification ensures the correct diagnosis.<sup>42</sup> Another way is to choose additional *S. aureus* or CoNS specific genes. These include typical *S. aureus* gene versions such as *femA*,<sup>42</sup> protein A (*spa*), coagulase (*coa*), and nuclease (*nuc*).<sup>43</sup> Other genes or gene combinations were also successfully used to discriminate between *S. aureus* and CoNS in clinical samples.<sup>42,44,45</sup>

Limits may occur with PCR amplification techniques. In some cases, proprietary DNA targets, known only to the manufacturer, can make it difficult to assess the vulnerability of commercial molecular assays to changes in the DNA sequence of isolates being tested. In a large multicenter US study of one molecular platform for identifying MRSA, 3 of 93 MRSA isolates were called methicillin-susceptible *S. aureus* (MSSA), and 8 of 102 MSSA isolates were called MRSA, indicating that although molecular typing may be useful for rapid screening of carriers, it may carry the risk of misdiagnosis in clinical care.<sup>46</sup> Likewise, PCR amplification using standard primers failed to amplify a new version of the *mecA* gene, renamed *mecC*, that emerged in livestock MRSA.<sup>47</sup> In this very case, methicillin resistance was detected with phenotypic tests.<sup>48</sup> Moreover, MSSA isolates have been found that had PCR-detected *mecA* elements but later reverted to methicillin resistance under therapy. The loss of a transposon that interrupted *mecA* and replication errors accounted for this conversion.<sup>49</sup>

Currently, more rapid identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is being developed, allowing MRSA identification from a colony within 5 minutes as compared with several hours with PCR.<sup>50</sup>

However, although more rapid than phenotypic tests, these molecular techniques still require prior growth of the organisms, which may take 12 to 24 hours, and do not test all possible antibiotic resistance genes. Whole-genome sequencing may become an option to screen for species and resistance genes, but the bioinformatic workload remains a limiting factor. One emerging technique relies on metagenomic analysis of DNA amplified directly from clinical samples, without prior culturing, through use of next-generation sequencing (NGS). Here, identification of genus, species, and known resistance genes will rely on comparison with large databases,<sup>51</sup> but unknown resistance genes will be missed. Thus, phenotypic testing must always be kept in mind.

## Molecular Typing

There is a dual interest in studying the genealogy of life. One is academic and aims at solving the evolutionary journey of peculiar organisms. The other is epidemiologic and aims at tracing a peculiar pathogen responsible for clinical problems. *S. aureus* is a common pathogen both in the hospital and in the community.<sup>27,52</sup> Although the prevalence of MRSA has been slowly decreasing over the last decade in the United States and Europe,<sup>53</sup> this trend is not global and the proportion of MRSA in health care-related infections remains over 50% in other geographic locations.<sup>54</sup>

MRSA is highly clonal, and a few highly successful clones, named according to the place where they were described, can be recovered at multiple locations both nationwide and worldwide (i.e., the Iberian, Brazilian, Hungarian, New York/Japan, Pediatric, and EMRSA-16 pandemic clones).<sup>55,56</sup> The main molecular typing methods underlying this comprehension are briefly presented later. More complete total

genome sequencing may not be required for routine tracing of epidemic strains.

## Pulsed-Field Gel Electrophoresis

The seminal method is a restriction-fragment length technique based on large chromosomal fragments generated by digestion with the low-frequency cutting enzyme *Sma*I. The fragments are separated with pulsed-field gel electrophoresis (PFGE) and yield banding patterns specific for particular clones. Banding comparison allowed identification of the major epidemic clones listed earlier, which represented 70% of more than 3000 MRSA isolates recovered worldwide.<sup>56</sup>

One limitation of PFGE is that it does not provide accurate information on the genealogy of the organism. Indeed, the length of chromosomal fragments, and thus the clone-specific banding, may be modified with acquisition or loss of mobile DNA (MGEs) such as transposons, prophages, or pathogenicity islands. The new banding pattern may identify a different clone, which is in fact the same bacterium that has gained or lost MGEs. This is exemplified by the fact that several PFGE major MRSA epidemic clones belonged to the same multilocus sequence typing (MLST; see later) group.<sup>55</sup> Thus, PFGE is useful to follow epidemic clones, but not to build the parental staphylococcal genealogy.

## Multilocus Sequence Typing

In contrast to PFGE, MLST is a sequence-based method that allows the unambiguous assignment of the ancestral phylogeny of the staphylococcal population.<sup>57</sup> It consists of sequencing seven housekeeping genes (i.e., *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) and comparing them with the sequences of other isolates collected in a central database ([www.mlst.net](http://www.mlst.net)). It compares allelic diversity based on approximately 500-bp internal gene fragments. Thousands of sequences have been submitted, generating numerous sequence types (STs). Organisms that share all seven alleles are defined as *clones*, those that share five of seven identical alleles are defined as *clonal complexes* (CC), and those that share less than five alleles are defined as *unrelated*. Within such arborescence, STs can be considered as founders of further evolutionary groups such as CCs.

Because housekeeping genes are independent of acquired MGEs, MLST traces staphylococci back to their latest common ancestor. Of the seven pandemic clones mentioned previously, six could be traced back to three ancestral MSSA types (i.e., CC5, CC8, and CC30; Fig. 194.2).<sup>58</sup> Thus, a few ancestral clones of MSSA took the lead and successfully colonized humans and animals before antibiotic resistance developed. Later acquisition of MGEs carrying drug-resistance or virulence genes helped further adaptation to new conditions (e.g., antibiotic use in hospitals), generating a new PFGE makeup on similar ancestral parents (see “Comparative Genomics and Evolution”). Moreover, genomics now shows that acquisition of antibiotic resistance genes is reversible and that the contemporary decrease in MRSA prevalence is associated with the loss of the methicillin-resistance determinants.<sup>53</sup>

## *Spa* Typing and Double-Locus

### *Spa-ClfB* Typing

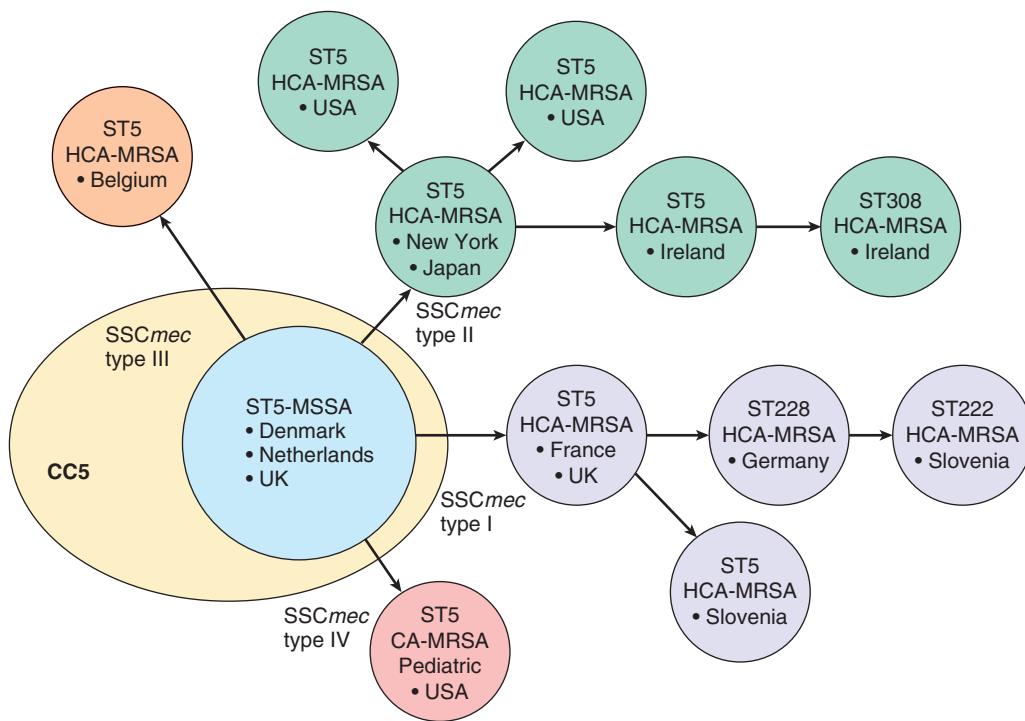
*Spa* typing and double-locus *spa-clfB* typing rely on PCR amplification of strain-specific regions within hypervariable segments of the *spa* (protein A) or *clfB* (clumping factor B) genes.<sup>59</sup> The variable regions are made of 24 nucleotide repeats in *spa* and serine-aspartate repeats in *clfB*, the length of which may vary from duplication or accidental loss of DNA material. Single PFGE or MLST types can evolve into different *spa* or *clfB* sublineages. Hence, combining these techniques generates unambiguous data sets that can be compared in multicenter studies.

Typing is critical in order to understand the *S. aureus* epidemiology. On the other hand, although a handful of founding ST types appear to be prevalent in MRSA<sup>55</sup> and PVL-positive strains,<sup>60</sup> no specific types could be attributed to disease-producing versus mere colonizing strains.<sup>10</sup>

## PATHOGENESIS

### Regulation and Virulence Determinants

*S. aureus* is extremely well equipped in surface factors and secreted proteins that mediate host colonization and disease (see Table 194.1).<sup>1</sup>



**FIG. 194.2 Evolution of methicillin-susceptible *Staphylococcus aureus* (MSSA) into methicillin-resistant *S. aureus* (MRSA) as exemplified by sequence type 5 (ST5).** ST5 belongs to clonal cluster 5 (CC5), which gathers *S. aureus* isolates sharing homologies in five of the seven genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqil*) compared with method of multilocus sequence typing (MLST). Parental ST5 is an MSSA that has been isolated in several countries, including Denmark, the Netherlands, and the United Kingdom. It acquired various types of SCCmec at several independent occasions, probably from coagulase-negative staphylococci (CoNS) donor strains. After SCCmec acquisition, new MRSA clones followed their own geographic and genetic evolution, spreading either as HCA-MRSA (SCCmec I, II, or III) or CA-MRSA clones (SCCmec IV) and sometimes evolving into new ST types (e.g., ST222, ST228, and ST308). Three clonal clusters (CC5, CC8, and CC30) generated six of the seven major pandemic MRSA clones described over the past 3 decades. (Modified from Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2003;47:3926–3934.)

In addition to these features, *S. aureus* is equipped with regulatory systems that sense environmental conditions and respond by fine-tuning the expression of given metabolic and virulence determinants (for review, see Novick and Geisinger,<sup>61</sup> Pragman and Schlievert,<sup>62</sup> and Balasubramanian and colleagues<sup>63</sup>). Some aspects of this subtle adaptation machinery are described subsequently.

## Regulation

At least three families of regulatory elements intertwine to adjust gene expression to specific environmental conditions: first, two-component regulatory systems, of which *agr* (for accessory gene regulator) is a paradigm; second, DNA-binding proteins, largely represented by the Sar (for staphylococcal accessory regulator) family of proteins; and third, small regulatory RNAs.

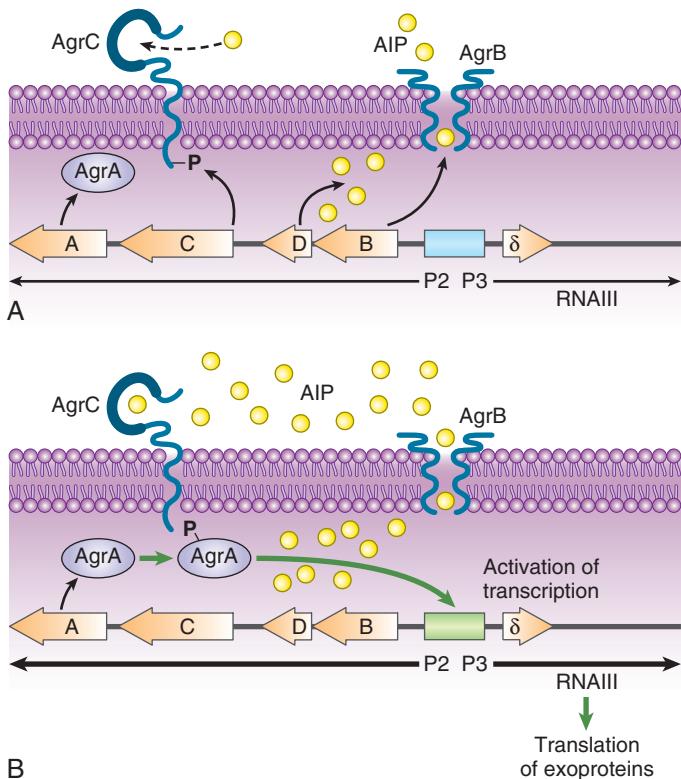
### *agr* and Other Two-Component Regulatory Systems

The paradigm of two-component regulatory systems virulence gene regulation is *agr*, which is schematized in Fig. 194.3.<sup>64</sup> *agr* functions as a quorum sensing control that reacts to bacterial density, allowing the preferential expression of surface adhesins during the exponential phase of growth (low cell density) and switching to the expression of exoproteins during the postexponential and stationary growth phases (high cell density).<sup>61,64,65</sup> The switch is composed of two divergent operons (see Fig. 194.3). On the left hand, promoter P2 drives the transcription of a series of components that comprises (1) a transmembrane protein (AgrB); (2) an autoinducing peptide precursor (AgrD), which is processed and exported by membrane-spanning AgrB; (3) a transmembrane sensor (AgrC), which is the cognate receptor of the AgrD-derived autoinducing peptide; and (4) a transcription regulator (AgrA) that can be activated by AgrC. At low cell density (exponential growth phase), the P2 promoter

is off and the operon is transcribed at a low level. As cell growth proceeds, the concentrations of both bacteria and extracellular autoinducing peptide increase in the milieu, thereby augmenting the chance of the autoinducing peptide to make contact with its cognate AgrC receptor. On contact, AgrC activates the response regulator AgrA, a process that may involve AgrA dephosphorylation.<sup>61,64</sup>

Activated AgrA is a DNA-binding protein that turns on the transcription from both promoter P2, generating a positive feedback on the system, and promoter P3, which drives the transcription of δ-hemolysin and of a peculiar effector called RNAIII. RNAIII has a reciprocal effect and activates the expression of several secreted proteins while downregulating the expression of surface-bound factors (see Table 194.1). RNAIII has a complex three-dimensional structure and a long half-life (up to 15 minutes). It regulates gene expression in several ways, including at the translational level by blocking the messenger RNA (mRNA) ribosome-binding site (RBS) of the target genes, or by prolonging the half-life of mRNA of downstream pleiotropic transcriptional regulators such as MgrA.<sup>66</sup>

The *S. aureus* chromosome encodes for up to 16 two-component regulatory systems involved in both metabolic environmental control and virulence gene regulation.<sup>61,64,65</sup> Important two-component regulatory systems regarding virulence genes include *saeR/S* (for *S. aureus* exoproteins),<sup>67</sup> *srrAB* (for staphylococcal respiratory response),<sup>68</sup> and *arlS* (for autolysis-related locus sensor).<sup>69</sup> *saeR/S* was identified with transposon mutation in a pleiotropic mutant defective in exoprotein synthesis other than that regulated by *agr* (e.g., coagulase and nuclease; see Table 194.1).<sup>70</sup> *saeR/S* acts independently of *agr* and responds to environmental stimuli such as high salt, low pH, glucose, and subinhibitory antibiotic concentrations. *srrAB* and *arlS* interfere with growth in microaerobic conditions and autolysis, respectively. *srrAB* represses the expression of TSST-1 and protein A in microaerobic conditions,<sup>68</sup> an observation that may be relevant for the pathogenesis of tampon-related



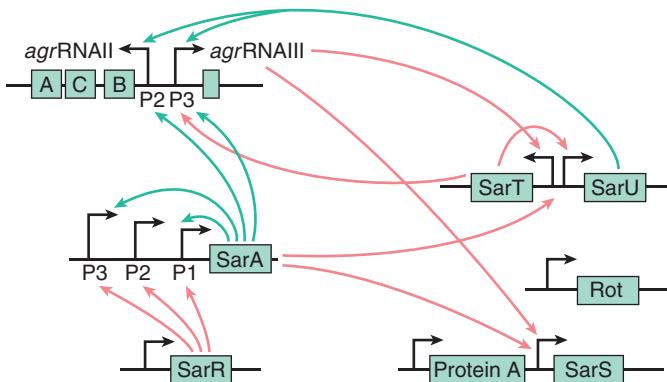
**FIG. 194.3 Schematic representation of *Staphylococcus aureus* global regulatory system *agr* (accessory gene regulator).** (A) System at rest. It consists of two divergent operons, transcribed from promoters P2 and P3. Promoter P2 encodes putative membrane protein AgrB, precursor of autoinducing peptide (AIP) AgrD, which is processed by AgrB; transmembrane receptor AgrC; and response regulator AgrA. Promoter P3 encodes  $\delta$ -hemolysin and RNAIII. At low bacterial density, P2 and P3 are off and only a small amount of AIP is secreted because of promoter leakiness (A). As bacterial growth proceeds and bacterial density increases (B), chance of AIP encountering its cognate receptor AgrC increases. On contact, AgrC undergoes conformational change and phosphorylates (or dephosphorylates) response regulator AgrA. (B) Activated AgrA activates transcription from both P2 and P3, resulting in positive feedback.  $\delta$ -Hemolysin is membrane-active protein toxic for eukaryotic cells. RNAIII is an intracellular regulator that acts in *trans* and regulates expression of many virulence genes, including numerous toxins (see Table 194.1). Although *agr* is pivotal in quorum-sensing regulation of gene expression, it is not the only regulator of pathogenic determinants in *S. aureus*. *sar*, *saerS*, *rot*, and other systems may affect the expression of *agr* itself or affect virulence genes directly (e.g., *sar*) or both (see Table 194.1).

TSS (see later discussion).<sup>5</sup> Both *srrAB* and *arlS* interact reciprocally with *agr*.<sup>62,65</sup>

#### DNA-Binding Proteins

*sar* is an important locus that encodes the DNA-binding protein SarA, which positively controls *agr* (Fig. 194.4), and maybe also *sae* and *arlS*.<sup>61,65</sup> In addition, *sar* directly regulates adhesin genes (see Table 194.1). The *sarA* transcripts peak at the end of the logarithmic phase of growth, thus promoting *agr* expression. Moreover, *sarA* itself is transcribed downstream of three alternate promoters, which can themselves be regulated by as yet incompletely solved factors.

SarA is the prototype of a growing family of DNA-binding proteins that may drive a number of transcriptional activities, including the expression of housekeeping genes and phage-related genes. *sara* homologues include *sarR*, *sarS*, *sarT*, *sarV*, *sarU*, *sarY*, *rot*, and *mgra*.<sup>62,65,71</sup> *rot* stands for “repressor of toxins” and counters toxin expression by repressing *agr*.<sup>72</sup> Inactivation of *rot* partially restored the *agr* phenotype in *agr*-negative mutants, probably by alleviating a repressing effect on the downstream P3 cascade of the *agr*. This downstream cascade might be the target of several additional regulators that also affect the *agr* phenotype (see Fig. 194.4). *mgra* stands for multiple gene regulator.<sup>71</sup>



**FIG. 194.4 Regulatory network of *agr* and *Sar* family of DNA-binding proteins.** Intertwining of activation (green arrows) and repression (red arrows) underlines complexity of system. Gene expression is further modulated by additional factors, including alternative  $\sigma^B$ , *arlS*, *sae*, and *srrAB*, which can act on *agr* promoters or directly on specific genes. Gene promoters are denominated P1, P2, and P3 and represented by black lines. (Modified from Pragman AA, Schlievert PM. Virulence regulation in *Staphylococcus aureus*: the need for *in vivo* analysis of virulence factor regulation. FEMS Immunol Med Microbiol. 2004;42:147–154.)

It controls the transcription of up to 355 genes (175 upregulated and 180 downregulated), including capsule, and protein A and  $\alpha$ -hemolysin genes in an *agr*-dependent way.<sup>66,71</sup>

Sigma factors ( $\sigma$ ) are another major mechanism of response to environmental stimuli. In bacteria,  $\sigma$  factors combine with and activate RNA polymerase to transcribe specific sets of genes. *S. aureus* contains one  $\sigma^A$  and two alternative  $\sigma^B$  and  $\sigma^C$ . Alternative  $\sigma^B$  is important for the microbial response to a variety of stresses, including temperature, energy depletion, and chemical stimuli.<sup>73</sup> It acts mostly via the global regulatory network and affects the expression of up to 251 genes (198 positively, 53 negatively),<sup>74</sup> but also has some direct effect by activating the expression of coagulase and fibronectin-binding proteins at the early growth phase, and downregulating certain secreted proteins in the stationary phase. Mutants overexpressing  $\sigma^B$  were more virulent in experimental endocarditis, probably by increasing the expression of surface adhesins.<sup>75</sup> Conversely  $\sigma^B$  defective mutants were less infective in a model of catheter-related systemic infection.<sup>76</sup>

#### Small RNAs and Endoribonuclease III

Small RNAs (sRNAs) are increasingly recognized as major players in regulation of gene expression. They act mainly at the translational level via antisense hybridization with mRNA, where they can alter mRNA stability, hide RBSs from ribosome recognition, or conversely reveal RBSs that are hidden in secondary mRNA structures by unfolding these very structures. Alternatively, sRNA can also bind regulatory DNA-binding proteins, thus sequestering them from their original gene regulatory function. A genome-wide analysis generated a “Staphylococcal Regulatory RNA Database” (SRD; <http://srd.genouest.org/>) and identified at least 550 potential regulatory sRNAs. The best functionally characterized of them are *RNAIII*, which orchestrates the *agr* response, and *RNAI*, which regulates the replication of multiresistance plasmid pSK41.

In symmetry, posttranscriptional expression is also modulated by direct RNA alteration via endoribonuclease III (RNase III). This RNA double-stranded endonuclease plays a critical role in RNA processing and decay. It has been shown to modulate posttranscriptional expression through various mechanisms, including turnover of transcribed and nontranscribed RNAs, and by maturing the 5' untranslated region (5'UTR) of the mRNAs of the cold-shock protein *cpsA* and maybe the protein A *spa* genes, to increase their stability and translation.<sup>77</sup>

The regulatory network must be considered as a metabolic hub that integrates both external and internal information and responds in the most appropriate way. The observed phenotypes result from complex interplays among sometimes contradicting signals of sensors and transcriptional and posttranscriptional regulators, the understanding of which will require a systems biology approach.<sup>78</sup> Moreover,

experimentally interrupting one of these circuitries may cause compensation by others, thus introducing biases in the observed phenotype. In this complex system, *agr* appears to be a central switch toward which many other regulators converge (see Fig. 194.4).

#### Role in Pathogenesis

The intuitive *agr*-based model suggests that scattered growing bacteria produce primarily adhesins, promoting tissue colonization, whereas installed organisms that form dense populations switch to the production of hydrolytic enzymes and toxins for the purpose of feeding and escaping host defenses.<sup>61,65</sup> Accordingly, inactivation of the function of *agr* alone decreased pathogenicity in experimental models of tissue destruction (e.g., subcutaneous abscesses), where exoprotein production is likely to be important.<sup>79</sup> On the other hand, *agr* inactivation did not much influence the course of experimental endocarditis, where bacterial surface adhesins are critical for valve colonization.<sup>80</sup> Indeed, although *agr*-negative mutants are hampered in exoprotein production, they are still fully equipped with surface-bound colonizing determinants (see Table 194.1). In contrast, inactivation of *sar* decreased infectivity in experimental endocarditis<sup>80</sup> because in addition to its effect on *agr* expression (see Table 194.1 and Fig. 194.4), *sar* also acts directly on expression of surface-bound fibronectin-binding protein A (FnBPA), which promotes experimental endocarditis.<sup>81,82</sup>

In addition, *in vivo* gene expression revealed a further level of complexity.<sup>62</sup> For instance, although *sar* transcripts were detected in infected vegetations during experimental endocarditis, they were expressed from both P1 and P2 promoters, rather than only from the P1 promoter as observed *in vitro*.<sup>83</sup> Likewise, *in vivo* expression of several genes appeared dissociated from their control regulator as described *in vitro*. Although *agr* positively regulates TSST-1 *in vitro* (see Table 194.1), the toxin was still expressed by an *agr*-negative mutant in a rabbit model of TSS *in vivo*.<sup>84</sup> This may result from alternative regulation by other regulators that act either downstream of the *agr* locus or directly on the *tss* gene promoter. Eventually, *agr*-negative mutants can be recovered from clinical samples as in cystic fibrosis<sup>85</sup> and in carrier and bacteremic patients.<sup>86</sup> Such *agr*-negative clinical isolates, and *agr*-negative laboratory mutants, have increased surface adhesins and an increased ability to form biofilms, and are found in chronic infections such as osteomyelitis and device infections.<sup>87</sup>

Hence, the pathogenic implication of regulatory circuitries cannot be drawn merely from *in vitro* observations. *In vivo* experimentation reveals the plurality of *S. aureus* infection forms, which may be variously altered by novel antivirulence therapies. For instance, inhibition of the *agr* loop by action on the autoinducing peptide impedes acute tissue destruction<sup>79</sup> but might promote biofilm formation and chronic infection.<sup>87,88</sup>

#### Ecologic and Epidemiologic Implication of *agr*

Genetic and functional experiments revealed the existence of at least four *agr* groups in *S. aureus*, which were characterized by specific variations in all three AgrB, AgrD, and AgrC proteins (see Fig. 194.3).<sup>89</sup> Whereas the autoinducing peptide of a given *agr* group stimulated signaling in other strains sharing the same *agr* group, it either cross-inhibited (e.g., group I and group IV) or cross-activated (e.g., group I and group II) members of other groups. This suggests that certain antagonistic *agr* groups could be mutually exclusive with attempts to simultaneously colonize the same niche. However, studies regarding this hypothesis gave conflicting results. In particular, patients with cystic fibrosis colonized with *S. aureus* can successfully harbor organisms from two antagonistic *agr* groups.<sup>90</sup>

Although *agr* and other global regulators control the timely expression of pathogenic genes, they are not bona fide pathogenic factors themselves. The *agr* locus has homologues in numerous nonpathogenic staphylococci. A phylogenetic study of nonpathogenic CoNS indicated that variations in *agr* genes followed parallel variations in species-specific rRNA genes.<sup>91</sup> In fact, *agr* groups diverged very early during the evolution of staphylococci (see “Comparative Genomics and Evolution”) and represent a lineage marker of strains that evolve in distinct environments rather than a strategy to exclude potential competitors. Thus, global regulators were originally meant to control the expression of useful metabolic genes. How exogenous virulent genes, which were acquired later,

succeeded in taking advantage of such systems remains a fascinating question of evolutionary genetics.

#### The Journey to Invasive Disease

Although *S. aureus* is an innocuous resident of the skin and mucosal flora in up to 30% of the human population,<sup>20–22</sup> healthy carriers are notoriously more prone than noncarriers to develop invasive *S. aureus* infections. This is exemplified by recurrent skin and wound or blood-stream infections (BSIs), which are due to the patient's own carriage strain in up to 80% of the cases.<sup>21,24</sup>

Colonization of the anterior nares is ideal for microbial dissemination. Outward dissemination is illustrated in Fig. 194.5. A few drops of fluorescein were instilled intranasally in a volunteer, followed by ultraviolet imaging. Two hours after instillation, fluorescein was all over the hands and clothes, ensuring both sneezing-induced and contact dissemination.

Inward dissemination by host invasion is an opportunity for ample bacterial proliferation. Host invasion is often considered a bacterial dead end, because invading microorganisms may be destroyed by the immune system. However, this only holds true if the immune system can eliminate the invading organisms, which is mostly not the case with *S. aureus* (see “Immune Evasion” later). Alternatively, invading microbes can kill the host, but then incur the risk of disappearing with the decaying corpse. Nevertheless, although this is expected in humans wherein dead bodies are eliminated through burial or cremation, it is different in the wild, where scavengers eat corpses, thus contributing to further dissemination. As a result, *S. aureus* has little evolutionary pressure to dampen its invasive lifestyle—which comes in addition to commensalism—whereas it has ample reasons to withstand host defenses, including resistance to antibiotics.

#### Mucosal and Skin Colonization

Persistent mucosal and skin colonization is critical. Factors involved in attachment to nasal epithelia involve teichoic acids,<sup>92</sup> which may attach



**FIG. 194.5 Example of environmental spread of bacteria colonizing the nose.** A few drops of fluorescein were instilled in the anterior nares of a volunteer, who was left to go about his routine activities and was photographed under ultraviolet light 2 hours later. Fluorescein is found all over face, hands, and clothes, exemplifying the appropriateness of colonizing the nose to ensure rapid microbial spread.

to lectin glycoproteins on the surface of mucosal cells, fibrinogen-binding protein B (clumping factor B or ClfB), serine-aspartate rich proteins C and D (SdrC and SdrD), SasG, and IsdA (Table 194.3).<sup>93–95</sup> ClfB, SdrC, SdrD, SasG, and IsdA are members of a family of *S. aureus* surface-bound proteins referred to as MSCRAMMs (for matrix surface components recognizing matrix molecules).<sup>96</sup> MSCRAMMs are covalently attached to the *S. aureus* peptidoglycan via the membrane-bound transpeptidase sortase A (SortA) (Fig. 194.6). SortA-attached proteins include at least 21 members,<sup>97</sup> some of them having functions different than host-matrix

adherence (see Table 194.3). However, all to them are recognized by SortA at an LPXTG signature motif, cleaved by SortA between LPXT and G and covalently attached to the last G (glycine) residue of the peptidoglycan pentaglycine side chain (see Fig. 194.6).<sup>98,99</sup>

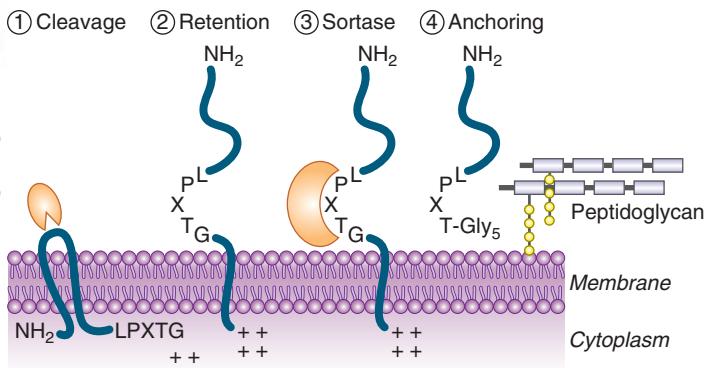
ClfB, SdrC, and SdrD are able to bind fibrinogen and keratin in vitro. SdrD was also shown to bind desmoglein 1, a desmosomal transmembrane protein that binds epidermal cells to keratin,<sup>100</sup> which is likely to facilitate *S. aureus* persistence in deeper layer of keratinized epithelia. The epithelial ligand of SasG is not known, but SasG is involved

**TABLE 194.3 *Staphylococcus aureus* MSCRAMMs Belonging to Sortase-Mediated Cell Wall-Associated Proteins**

GENE	PROTEIN	AA	SORTASE	MOTIF	LIGAND SPECIFICITY	POTENTIAL IMPLICATION IN DISEASE
Spa	Protein A	508	SrtA	LPETG	Antibody Fc fragment (IgG, IgM) von Willebrand factor, TNFR1, platelets	Experimental sepsis, experimental osteoarthritis
clfA	Clumping factor A	933	SrtA	LPDTG	Fibrinogen, platelets	Experimental endocarditis
clfB	Clumping factor B	913	SrtA	LPETG	Fibrinogen, cytokeratin 10, platelets	Colonization of nasal mucosa
cna	Collagen-binding protein	1183	SrtA	LPKTG	Collagen	Experimental osteomyelitis, septic arthritis
fnA	Fibronectin-binding protein A	1018	SrtA	LPETG	Fibronectin, fibrinogen, elastin Platelets	Experimental endocarditis Cell invasion, experimental mastitis
fnB	Fibronectin-binding protein B	914	SrtA	LPETG	Fibronectin, fibrinogen, elastin, platelets	Experimental mastitis
sdrC	Serine-aspartate repeat protein	947	SrtA	LPETG	Fibrinogen, cytokeratin of nasal epithelia	Nasal colonization
sdrD	Serine-aspartate repeat protein	1315	SrtA	LPETG	Fibrinogen, desmosomal desmoglein	Nasal, deep skin colonization, biofilm
sdrE	Serine-aspartate repeat protein	1166	SrtA	LPETG	Bridges fibrinogen and complement factor H on the <i>S. aureus</i> surface	Immune evasion
pls	Plasmin-sensitive protein	1637	SrtA	LPDTG	Cellular lipids, ganglioside M3; nasal epithelial cells	Colonization of nasal mucosa
sraP (or sasA)	Serine-rich adhesin for platelets	2261	SrtA	LPDTG	Platelets	Experimental endocarditis
IsdA	Iron-regulated surface determinant A (sasE)	354	SrtA	LPKTG	Fibrinogen, fibronectin Hemoglobin/transferrin	Nasal colonization
IsdB	Iron-regulated surface determinant B (sasJ)	645	SrtA	LPQTG	Hemoglobin/hemin	Experimental bacteremia and renal abscesses
isdC	Iron-regulated surface determinant C	227	SrtB	NPQTN	Hemin	Experimental bacteremia and renal abscesses
isdH (or haR)	Iron-regulated surface determinant H	895	SrtA	LPKTG	Haptoglobin/hemoglobin complex	Nasal colonization
sasl	Putative <i>S. aureus</i> surface protein I				Undetermined	Associated with bovine gangrenous mastitis strains
sasB	<i>S. aureus</i> surface protein B	937	SrtA	LPDTG	Undetermined	—
sasC	<i>S. aureus</i> surface protein C	2186	SrtA	LPNTG	Intercellular adhesion	Involved in biofilm
sasD	<i>S. aureus</i> surface protein D	241	SrtA	LPAAG	Undetermined	Involved in biofilm
sasF	<i>S. aureus</i> surface protein F	637	SrtA	LPKAG	Undetermined	—
sasG	<i>S. aureus</i> surface protein G	1117	SrtA	LPKTG	Nasal epithelial cells	Associated to invasive disease
Sas (or adsA)	<i>S. aureus</i> surface protein H	308	SrtA	LPKTG	Cell wall associated adenosine synthase	Escape phagocyte-induced killing
sasK	<i>S. aureus</i> surface protein K	211	SrtA	LPKTG	Undetermined	—
fmtB	Formyl transferase B		SrtA	LPXTG	Cell wall synthesis, $\beta$ -lactam resistance	Antibiotic resistance

AA, Protein length in amino acids; IgG, immunoglobulin G; IgM, immunoglobulin M; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; Srt, sortase; TNFR1, tumor necrosis factor receptor 1.

Modified from Roche FM, Massey R, Peacock SJ, et al. Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. *Microbiology*. 2003;149:643–654; Clarke S, Foster S. Surface adhesins of *Staphylococcus aureus*. *Adv Microb Physiol*. 2006;51:187–224; and Dedent A, Marraffini L, Schneewind O. Staphylococcal sortases and surface proteins. In: Fischetti V, Novick RP, Ferretti J, et al, eds. *Gram-Positive Pathogens*. 2nd ed. Washington, DC: ASM Press; 2006:486–495.



**FIG. 194.6 Anchoring of gram-positive surface proteins to peptidoglycan through sortase-mediated processing of LPXTG consensus motif.** During membrane translocation, amino-terminal leader sequence is clipped off. Protein is then transiently retained on cell surface through membrane-anchor domain, rich in positively charged amino acids at its intracellular carboxyl-terminal portion. LPXTG consensus region is then processed by sortase that clips between threonine and glycine (T-G) and transfers covalent bond to glycine acceptor in peptidoglycan meshwork. (Modified from Fischetti VA, Pancholi V, Schneewind O. Conservation of a hexapeptide sequence in the anchor region of surface proteins from gram-positive cocci. Mol Microbiol. 1990;4:1603–1605; Mazmanian SK, Ton-That H, Schneewind O. Sortase-catalysed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. Mol Microbiol. 2001;40:1049–1057; and Bradshaw WJ et al. Molecular features of the sortase enzyme family. FEBS J. 2015;282:2097–2114.)

in biofilm formation, which is an ingredient of local persistence.<sup>101</sup> Likewise, the exact role of IsdA in nasal colonization is unclear. However, because IsdA is a heme-binding protein,<sup>102</sup> it might help acquire essential iron for the colonizing bacteria.<sup>95</sup> Finally, *S. aureus* can also survive in a dormant state inside nasal epithelial cells.<sup>103</sup> This is one reason, in addition to biofilm, that explains why it is difficult to eradicate chronic carriage, especially with antimicrobials that do not penetrate inside eukaryotes cells—for instance,  $\beta$ -lactams.

Whether the fibrinogen-binding capacity of ClfB, SdrC, and SdrD may facilitate further invasion in case of mucosal or skin breaches is not known. On the other hand, SasG (among few others) elicited an antibody response in patients with invasive *S. aureus* diseases,<sup>97</sup> a finding that supports its involvement in deep-seated infections.

Most interestingly, there is an as yet unexplained privileged liaison between *S. aureus* and chronic nasal carriers. In one study, *S. aureus* carrier and noncarrier volunteers had the nose disinfected and reinoculated with a mixture of four *S. aureus* strains, including the carriage strain in case of chronic carriers. Over a few days, noncarriers tended to eliminate all inoculated strains, whereas chronic carriers eliminated all foreign strains and reselected their own.<sup>104</sup>

In certain occurrences, mucosal or wound colonization with *S. aureus* may produce distant diseases such as SSSS or TSS. These issues are discussed in dedicated sections later.

Taken together, although the subtle relation between *S. aureus* and its host remains incompletely solved, it is critical to detect and eliminate *S. aureus* carriage in groups at risk of severe infection, such as patients undergoing operation,<sup>26</sup> dialysis patients,<sup>105</sup> and possibly patients with prosthetic heart valves, in whom the 1-year mortality of *S. aureus* valve infection is up to 50%.<sup>106</sup>

### Host Invasion

Because *S. aureus* is nonmotile, invasion takes advantage of mucosal or skin breaches, where the microorganism engages with constituents of deeper tissue and blood compartments. The first encounters are constituents of microthrombi, which occur as a normal healing process of tissue breaches. *S. aureus* avidly binds to soluble fibrinogen and clotted fibrin via clumping factor A (ClfA)<sup>107</sup> and the fibrinogen-binding domain of fibronectin-binding protein A (FnBPA).<sup>108</sup> ClfA and FnBPA are SortA-LPXTG wall-associated MSCRAMMs (see

Fig. 194.4 and Table 194.3), that serve at least two purposes. On one hand, they encourage *S. aureus* attachment at the place of preexisting lesions.<sup>81</sup> On the other hand, they also act as immune camouflage factors against complement-induced phagocytosis by surrounding the bacterium with a shield of soluble fibrinogen and fibronectin (see Table 194.3).<sup>109</sup>

Once attached to microthrombi, *S. aureus* may encourage further thrombus formation via the action of two secreted coagulases—coagulase (Coa) and von Willebrand-binding protein (vWbp). Coa and vWbp bind to prothrombin and induce a conformational change that converts it into active staphylothrombin.<sup>110</sup> Staphylothrombin is unique in that it polymerizes fibrinogen into fibrin and activates platelets even in blood anticoagulated via coumarin therapy, or exposed to heparin or calcium chelators.

Then, in order to avoid local trapping and hamper further spread, *S. aureus* needs to control local coagulation. To this end, coagulase is only transiently produced in the early exponential phase of growth. Moreover, *S. aureus* can escape clots by secreting staphylokinase (Sak), a protease that activates host plasminogen into active plasmin, which in turns disintegrates fibrin clots<sup>111</sup> and promotes extension of local infections.<sup>112</sup> Sak also cleaves complement opsonin C3b and preformed antibodies, contributing to the antiphagocytic properties of *S. aureus* (see “Immune Evasion” later). Sak is produced in both the early exponential and the late stationary growth phases. Its gene (*sak*) is located, together with SCIN (for staphylococcal complement inhibiting protein) and CHIPS (for chemotaxis inhibitory protein of *Staphylococcus*), on a so-called immune escape cluster (IEC) as part of a  $\phi$ Sa3  $\beta$ -hemolysin-converting prophage, which is present in >90% of human *S. aureus* isolates, but usually not in animal isolates.<sup>113,114</sup> Thus, the  $\phi$ Sa3 prophage and its IEC cargo are believed to participate to the *S. aureus* specificity for human hosts.

### Contribution of Coagulation

It was originally thought that *S. aureus* surface MSCRAMMs would mediate direct binding to ligands present in target organs, such as binding to collagen via collagen-binding protein (Cna) in osteoarthritis.<sup>115,116</sup> However, in the case of hematogenous dissemination, reaching the target organ requires prior *S. aureus* extravasation from the microcirculation. It was shown that ClfA and FnBPA are critical to colonize and invade damaged or inflamed endothelia, and this presumably occurred by direct attachment to the injured tissues.<sup>81</sup> However, in the bloodstream, ClfA and FnBPA become rapidly saturated with soluble fibrinogen and fibronectin, which interfere with direct binding to injured vessels.<sup>109</sup> Thus there is a missing link, which *S. aureus* circumvent by hijacking the coagulation system.<sup>117</sup>

While circulating *S. aureus* organisms become saturated with soluble fibrinogen and fibronectin, ClfA and FnBPA induce a fibrinogen conformational change that triggers its docking to the platelet GPIIb/IIIa receptor and activates platelets.<sup>118</sup> In addition, preexisting anti-ClfA or anti-FnBPA antibodies, if present, activate platelets by docking to the immunoglobulin G (IgG) platelet receptor Fc $\gamma$ RIIA. These microaggregates are then conveyed through the blood to inflamed endothelia or to nascent platelet-fibrin clots present on injured tissues, to which activated platelets attach.

The convergence of these *S. aureus*-platelet microaggregates to inflamed endothelia is further strengthened by the second staphylococcal coagulase vWbp.<sup>110</sup> vWbp has the dual capacity to activate blood coagulation and to bind endothelial-attached von Willebrand factor (vWF). vWF is secreted as monomer by inflamed endothelia or activated platelets. vWF monomers attach to injured tissues and polymerize into discrete strings floating in the vascular flow, which bind activated platelets and microaggregates in a sheer-dependent manner.<sup>119</sup> *S. aureus*-secreted vWbp binds on one hand to the floating WWF strings, and on the other hand to *S. aureus*-attached ClfA,<sup>120</sup> thus also favoring the halt of circulating *S. aureus* onto inflamed endothelia.

This platelet-staphylothrombin scenario was validated in experimental models of endovascular colonization and endocarditis, in which prophylaxis with antiplatelet (acetylsalicylic acid and ticlopidine) or antithrombin (dabigatran) agents successfully prevented *S. aureus* endovascular infections.<sup>121,122</sup> In contrast, coumarin anticoagulation,

which relies on a different mechanism, did not prevent experimental endovascular infection.

Of note, platelet activation may be a double-edged sword in that platelet degranulation produces platelet-microbial peptides (PMP) that destabilize bacterial membranes and can kill bacteria.<sup>123</sup> However, *S. aureus* strains that produce successful endovascular infection are known to resist PMP-induced killing via plasma membrane modification.<sup>124,125</sup>

Eventually, inflamed endothelial cells also express  $\alpha 5\beta 1$  integrins, which bind soluble fibronectin and normally act as a landing runway for neutrophils. However,  $\alpha 5\beta 1$ -bound fibronectin also promotes *S. aureus* attachment via FnBPA, which triggers active bacterial internalization by endothelial cells.<sup>81</sup> Local tissue destruction ensues, and more specific molecules can enter into action, including specific MSCRAMMs (e.g., Cna),<sup>115,116</sup> biofilm facilitating factors,<sup>126</sup> hemolysins, and immune evasion molecules.

### Immune Evasion

Along with invasion and tissue colonization, *S. aureus* has to confront several layers of host defenses against which it applies an extremely sophisticated immune evasion armamentarium, which is briefly described in the following sections (Table 194.4) (for review, see Foster,<sup>9</sup> Kim and colleagues,<sup>127</sup> and Guerra and colleagues<sup>128</sup>).

#### Escaping Phagocytosis

The first line of anti-*S. aureus* host defense is phagocyte engulfment, primarily by neutrophils, either by direct recognition of pathogen-associated molecular patterns (PAMPs),<sup>129</sup> or via complement-mediated opsonization. Direct PAMP recognition is hampered by the production of polysaccharidic capsules (mostly type 5 or 8 in human *S. aureus* isolates), which are not recognized by professional phagocytes and physically block their access to underlying PAMPs, such as teichoic acids, lipoteichoic acids, peptidoglycan, and even C3b complement opsonins attached to these PAMP structures.

In addition, *S. aureus* uses several secreted and SortA-LPXTG anchored surface factors to counters phagocytosis. Secreted factors include the chemotaxis inhibitory protein CHIPS<sup>111</sup> and the extracellular adherence protein Eap (or Map).<sup>130</sup> CHIPS belongs to the  $\phi Sa3$  prophage IEC and blocks the neutrophil receptor for formyl-peptides, a universal signature of bacterial protein synthesis, and the C5a receptor for chemotaxis.<sup>111</sup> Eap binds intercellular adhesion molecule 1 (ICAM-1) and fibrinogen and vitronectin, and blocks leukocyte adhesion and neutrophil recruitment mediated by  $\beta 2$ -integrin and urokinase receptors in vitro and in vivo.<sup>130</sup>

SortA-LPXTG anchored molecules include protein A (Spa), ClfA, and adenosine synthase A (AdsA, also called SasA) (see Tables 194.3 and 194.4). Spa blocks antibody-mediated phagocytosis by binding IgGs by their Fc fragments and exposing the Fab fragments instead, which are not recognized by complement.<sup>127</sup> ClfA interferes with phagocytosis in a fibrinogen-dependent manner (probably by bacterial shielding) and an as yet unclear fibrinogen-independent manner.<sup>131</sup> AdsA converts adenosine monophosphate into adenosine, a dual immuno-modulator compound that has proinflammatory antiinflammatory properties.<sup>132</sup> *S. aureus*-generated adenosine was shown to impede neutrophil-mediated bacterial clearance and to promote abscess formation in a mouse model of sepsis and kidney abscess.<sup>133</sup>

#### Luring Complement

If not directly triggered by PAMPs, phagocytosis may be promoted by complement-mediated opsonization. The lectin and the alternative complement pathways are mainly involved against *S. aureus*. The classical pathway, which requires prior antibodies, is largely hampered protein A (Spa) and Sak, as mentioned earlier,<sup>9</sup> and by secreted staphylococcal binder of immunoglobulin (Sbi).<sup>134</sup> Sbi is both secreted and loosely attached to the bacterial envelope. Its envelope-attached form binds immunoglobulin Fc fragments similarly to Spa, and its secreted form binds complement factor H and C3d, which accelerate the decay of preopsonin C3. Soluble Sbi-C3d-factor H complexes also bind the complement receptor CR2 of B lymphocytes, promoting their apoptosis and impeding antibody production.<sup>127</sup> In addition, direct complement-induced bacterial killing via the C8-C9 polymerization membrane attack

complex (MAC) is not effective against gram-positive bacteria, because their plasma membrane is physically protected from MAC by the thick peptidoglycan cell wall (for review see Zipfel<sup>135</sup>).

The lectin and alternative complement pathways are triggered by PAMPs, which activate the lectin or alternative pathway-dependent convertases C4b2a and C3bBb. The convertases cleave C3 into C3a, which amplifies the chemoattractant loop, and C3b, which binds to staphylococcal teichoic acids and attracts phagocytes.<sup>135</sup> *S. aureus* counteracts complement-mediated opsonization by means of several mechanisms. First, as mentioned earlier, it can produce polysaccharidic capsules, hindering phagocyte access to teichoic acid-attached C3b. Second, it secretes Sak, which cleaves C3 and C3b. Third, it produces staphylococcal complement inhibitory protein SCIN, which binds to and inhibits the C4b2a and C3bBb convertases, thus blocking the production of C3a and C3b.<sup>111</sup> Like Sak, SCIN and CHIPS are located on the  $\phi Sa3$  prophage EIC.<sup>113</sup> They are expressed in the exponential phase of growth, whereas Sak is also expressed later in the late stationary growth phase.<sup>111</sup>

A fourth mechanism involves secreted extracellular fibrinogen binding protein (Efb), a dual adhesin capable of binding bacterial-attached C3b proximally, and soluble fibrinogen distally. As a result, Efb contributes an additional external fibrinogen shield, preventing the contact of neutrophils with C3b.<sup>136</sup>

Finally, a most astounding host-hijacking mechanism is conferred by SortA-LPXTG anchored SdrE. In addition to binding fibrinogen in vitro, SdrE binds to and attracts complement factor H on the *S. aureus* surface.<sup>137</sup> Factor H is a complement regulatory protein that normally binds to host cells and accelerates the decay of C3b in order to protect them from nonspecific assaults from self-host defenses. By attracting factor H on the *S. aureus* surface, SdrE usurps the complement host control system to its advantage.

#### Resisting Oxidative Burst

Activated neutrophils trigger oxidative burst and bacterial killing via NADPH oxidase and myeloperoxidase (MPO), or via nitric inducible oxide synthase (iNOS).<sup>138</sup> The cascade uses superoxide ( $O_2^-$ ) to produce highly oxidative molecules such as  $H_2O_2$  or hypochlorous acid (HOCl). Oxidation results in protein, lipid, and nucleic acid damage that can kill pathogens either extracellularly or inside phagolysosomes. Extracellular oxidative burst is exemplified by the neutrophil extracellular traps (NETs), which are constituted of neutrophil granules and chromatin proteins and contain up to 80% of neutrophil-released MPO.<sup>139</sup> *S. aureus* can disable these mechanisms by reducing enzymes such as catalase, which converts  $H_2O_2$  to water and  $O_2$ , or direct rescue of oxidized molecules by means of several reducing agents listed in Table 194.4.

#### Resisting Antimicrobial Peptides

Insects and animals produce an array of antimicrobial peptides (AMPs) consisting most often of 20–100-amino acid pore-forming  $\beta$ -sheet structures.<sup>140</sup> Human produces various AMPs in skin and mucosal tissues, and large quantities that are stored in granules of neutrophils and platelets (see “Contribution of Coagulation” earlier). A hallmark of these AMPs is that they are positively charged and are attracted by the negatively charged wall teichoic acids (Fig. 194.7) and membrane phospholipids of the gram-positive bacterial envelope. *S. aureus* modulates its susceptibility to AMPs by modulating its surface charge, either by means of the D-alanine lipoteichoic acid ligase (*dlt*) operon, which decorates teichoic acids with alanine residues, or by means of a lysyltransferase that transfers lysine residues to membrane phospholipids.<sup>141,142</sup> Both mechanisms result in a more positively charged bacterial envelope and thus in AMP repulsion. In particular, successful endocarditis *S. aureus* strains were shown to be consistently resistant to platelet-secreted PMPs,<sup>125</sup> a property that may discourage the development of AMPs for therapeutic purposes.

#### Killing Leukocytes

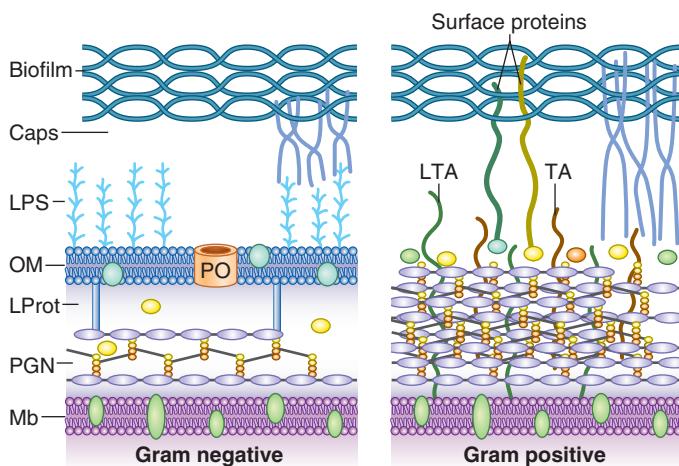
*S. aureus* kills eukaryotic cells via secreted hemolysins, leukocidins, and phenol-soluble modulins (PSMs).<sup>11,143,144</sup> There are four types of hemolysins, referred to as  $\alpha$ -hemolysin (Hla),  $\beta$ -hemolysin (Hlb),  $\delta$ -hemolysin (Hld), and  $\gamma$ -hemolysin (Hlg).

**TABLE 194.4 Main Immune Evasion Determinants**

INTERFERENCE WITH	DETERMINANT	LOCATION	FREQUENCY IN <i>STAPHYLOCOCCUS AUREUS</i> ISOLATES	ACTION
Neutrophil chemotaxis, migration and phagocytosis	Polysaccharidic capsule (mainly types 5 and 8)	Core genome	20%–60%	Not recognized as PAMPs Steric blockage of neutrophil access to deeper cells wall structures including wall attached C3 and C3b
	CHIPS (chemotaxis inhibitory protein of <i>Staphylococcus</i> )	φSa3 IEC	>60%	Blocks neutrophil C5a receptor Blocks neutrophil formyl-peptide receptor
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Interferes with neutrophil migration and extravasation by blocking the docking of neutrophil LFA-1 to endothelial ICAM-1 (See anticomplement activity below)
	AdsA (or Sash) (adenosine synthase A)	Core genome	70%–80%	Converts adenosine monophosphate to adenosine Interferes with inflammation and phagocytosis
Complement	SdrE (serine-aspartate repeat protein)	Core genome	40%–60%	Antiopsonic Binds factor H on the <i>S. aureus</i> surface
	SCIN	φSa3 IEC	>60%	Antiopsonic Inhibits lectin and alternative complement pathways Binds to and inhibits the C3 convertases C4b2 and C3bBb, thus blocking the generation of the C3b opsonin
	Sak (staphylokinase)	φSa3 IEC	>60%	Antiopsonic Converts plasminogen into plasmin which cleaves fibrin (solubilizing clots), IgGs, and bacterial attached C3b (See anti-AMP activity below)
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Antiopsonic Inhibits classical and lectin complement pathways Binds to and inhibits C3 convertase C4b2 and further C3b-mediated opsonization
	Efb (extracellular fibrinogen-binding protein)	IEC2	60%–70%	Antiopsonic Binds C3 components and fibrinogen Binds to staphylococcal wall-attached C3b and attracts plasma fibrinogen over it, thus shielding it from recognition by neutrophils
	Ecb (Extracellular complement binding protein)	IEC2	>95%	Antiopsonic Blocks binding of the neutrophil complement receptor CR1 to bacterial-attached C3b, thus preventing phagocytosis
Oxidative burst	SodA and SodM (super oxide dismutases)	Core genome		Convert superoxide radicals to H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> (using manganese as a co-factor)
	KatA (catalase)	Core genome		Converts H <sub>2</sub> O <sub>2</sub> into H <sub>2</sub> O and O <sub>2</sub>
	AhpC and AhpF (alkyl hydroperoxide reductases)	Core genome		Convert H <sub>2</sub> O <sub>2</sub> to H <sub>2</sub> O and O <sub>2</sub> Convert alkyl hydroperoxides to alcohol and water
	bNOS (bacterial nitric oxide synthase)	Core genome		Scavenges HOCl (hypochlorous acid).
	Bacillithiol	Core genome		Protection by S-thiolation of oxidants
	Coenzyme A Staphyloxanthin			Antioxidant mechanism unclear
Antimicrobial peptides (AMPs)	Dlt (D-alanine (lipo)teichoic acid ligase)	Core genome	>95%	Neutralizes negatively charged wall surface by alanine substitution of ribitol teichoic and lipoteichoic acids
	MprF (muropeptide resistance factor)	Core genome	>95%	Decreases surface affinity for positively charged AMPs Idem by adding L-lysine residues to phosphatidylglycerol at the extracellular side of the plasma membrane
	Sak (staphylokinase)	φSa3 IEC		Proteolytic degradation of fibrin (via plasmin activation), C3 components, IgGs, and AMPs
	Aur (aureolysin)	Core genome		Proteolytic cleavage of cathelicidin AMPs
Leukocyte lysis	Hla (α-hemolysin)	Core genome		Forms heptamer barrels in the plasma membrane of target cells
	Hlg (γ-hemolysin)	Core genome	>95%	Bicomponent leukocidin Lyses both erythrocytes and leukocytes
	Luk E/D (leukocidin E/D)	Genomic island beta	30%–40%	Synergohymenotropic bicomponent leukocidin
	Luk F/M (leukocidin F/M)	φSa1	2%–4%	Synergohymenotropic bicomponent leukocidin
	Panton-Valentine leukocidin	φSa2	>95%	Idem phenol-soluble modulins (PSMs) below
	Hld (delta-hemolysin)	Core genome	100%	Phagocyte lysis by membrane destabilization
	PSM alpha 1–4 PSM beta-1 and 2 (phenol-soluble modulins)	Core genome		Mechanism analogous to the delta-hemolysin mechanism of membrane damage

AMPs, Antimicrobial peptides; ICAM-1, intercellular adhesion molecule 1 (ligand of LFA-1); IEC, immune escape cluster; IgG, immunoglobulin G; LFA-1, lymphocyte function-associated antigen h1; MAP, major histocompatibility complex class II analogous protein; PAMPs, pathogen associated molecular patterns; φSa1 to φSa3, *Staphylococcus* prophages 1 to 3; Sash, *Staphylococcus* surface protein H; SCIN, staphylococcal complement inhibitory protein.

Modified from McCarthy AJ and Lindsay JA. *Staphylococcus aureus innate immune evasion is lineage-specific: a bioinformatics study*. *Infect Genet Evol*. 2013;19:7–14; Lindsay JA. *S. aureus evolution: lineages and mobile genetic elements (MGEs)*. In: Lindsay J, ed. *Staphylococcus aureus Molecular Genetics*. Norfolk, UK: Casper Academic Press; 2008:45–69; and Peakcock SJ, et al. *Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus*. *Infect Immun*. 2002;70:4987–4996.



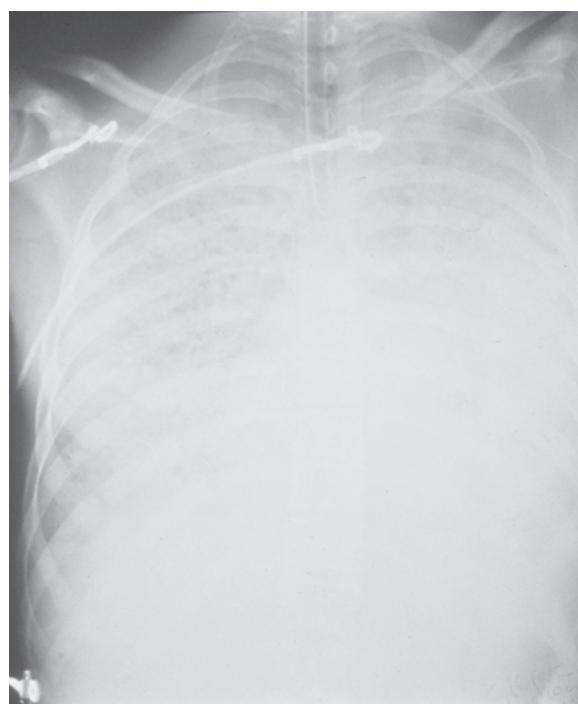
**FIG. 194.7 Schematic representation of gram-negative (left) and gram-positive (right) bacterial envelopes.** Gram-negative bacteria have very thin peptidoglycan (PGN) and an outer membrane (OM), made of lipopolysaccharide (LPS), which are not present in gram-positive bacteria. Peptidoglycan is very conserved and constituted of glycan chains made of N-acetylglucosamine and N-acetylmuramic acid disaccharide subunits, in which the N-acetylmuramate moiety is linked to highly conserved pentapeptide or tetrapeptide stems (L-alanine-D-isoglutamine-L-lysine-D-alanine-D-alanine). The chains of disaccharide peptide are cross-linked via peptide bridges between the penultimate D-alanine and the diamino acid L-lysine located in position 3 of a neighboring stem peptide. In *S. aureus*, the interpeptide bridge typically contains a polyglycine linking piece that comprises one to five glycine residues. The addition of glycines to the wall precursors is driven by the *femABC* and *fmhB* genes (see Fig. 194.13). Teichoic acids (TA) represent up to 50% of the dry weight of purified staphylococcal walls and are made of polyribitol-phosphate polymers cross-linked to N-acetylmuramic acid residues of the peptidoglycan and decorated with D-alanine and N-acetylglucosamine residues. Lipoteichoic acids (LTA) are the plasma membrane-bound counterparts of teichoic acids. They are made of polyglycerol phosphates chains and are linked to a diacylglycerol moiety that serves as a plasma membrane anchor. TA and LTA are not present in gram-negative bacteria. Caps, Capsule; Lprot, lipoprotein; Mb, plasma membrane; PO, porin. (Modified from Gotz F, Bannerman T, Schleifer K-H. The genera *Staphylococcus* and *Macrococcus*. In: Balows A, Truper AG, Dworkin M, et al, eds. *The Prokaryotes*. Vol. 4. New York: Springer Science+Business Media; 2006:41–75.)

Hla and Hld are secreted in nontoxic soluble forms and multimerize on eukaryotic membranes to form lytic pores.  $\alpha$ -Hemolysin (or  $\alpha$ -toxin) is involved in a great variety of diseases.<sup>145,146</sup> It multimerizes as heptamers on phosphocholine-containing membranes, a process which depends on the presence of host cell transmembrane protein ADAM10, which reunites both metalloprotease and disintegrin (integrin-binding) properties.<sup>147</sup> Moreover,  $\alpha$ -hemolysin interferes with adherens junction proteins to induce cell killing, most notably plekstrin-homology domain-containing protein 7 (PLEKHA7). Indeed, PLEKHA7-deficient cells can readily recover from Hla cytotoxicity.<sup>148</sup> Hence, polymorphism in this determinant could influence individual susceptibility to infection.

Hld acts in a similar way and belongs to the same family as PSMs, which were described more recently.<sup>144</sup> There are four PSM $\alpha$  types (PSM $\alpha_{1-4}$ ), consisting of approximately 20 amino acids, and two PSM $\beta$  types (PSM $\beta_{1-2}$ ), consisting of approximately 40 amino acids, the genes for which are located on the staphylococcal chromosome. Hld is located upstream of *agr* RNAIII regulatory RNA (see “Regulation” section). The structure of Hld and PSMs consists of amphipathic  $\alpha$ -helices, which confer several properties aside from membrane cell damage, including biofilm turnover and inflammatory responses.<sup>149</sup>

Hlb is distinctive because it is a sphingomyelinase that damages membranes by means of enzymatic alteration of their lipid content.

Hlg is also peculiar in that it is composed of two types of proteins called S and F, for slow and fast elution at chromatography. It promptly



**FIG. 194.8 Fulminant hemorrhagic pneumonia in 20-year-old patient infected with Panton-Valentine leukocidin-producing *Staphylococcus aureus*.**

lyses white blood cells in addition to other cells and is sometimes referred to as leukocidin. It is encoded by two distinct operons, one that encodes a unique HlgA (S protein) and another that encodes for one S protein (HlgC) and one F protein (HlgB). S and F proteins must assemble to form membrane-perforating complexes. Therefore, this class of hemolysins is also referred to as synergohemolytic toxins. Active  $\alpha$ -Hemolysin exists in two bioactive forms: HlgA-HlgB and HlgA-HlgC.

**Panton-valentine leukocidin.** PVL is a peculiar homologue of Hlg, which was originally reported in 1932 by Panton and Valentine.<sup>150,151</sup> PVL is encoded by two genes, *lukS* and *lukF*, which can assemble either between themselves or with the components of Hlg, thus producing chimera complexes. Like the other hemolysins, PVL is regulated by *agr* (see Table 194.1). Unlike the other hemolysins, PVL is encoded by mobile phages, including  $\phi$ SLT,  $\phi$ Sa2958,  $\phi$ Sa2MW,  $\phi$ PVL,  $\phi$ 108PVL,  $\phi$ 7247PVL,  $\phi$ Sa119,  $\phi$ TCH60, and  $\phi$ Sa2USA, which can transfer PVL to other strains.<sup>151,152</sup> Also unlike the other hemolysins, the prevalence rate of PVL is usually low ( $\leq 2\%$ ) in MSSA and health care-associated MRSA (HCA-MRSA),<sup>150</sup> whereas it is present in almost 100% of isolates of the community-acquired MRSA (CA-MRSA) USA300 cluster, which is peculiarly prevalent in North America.<sup>151,153</sup>

PVL-producing *S. aureus* is associated with skin and soft tissue infection (SSTI) and severe hemorrhagic pneumonia in children and young adults.<sup>154</sup> In contrast, it is rarely responsible for other infections, such as osteomyelitis, septicemia, and endocarditis. The reason for clustering in young patients is unclear. The clustering could be linked to an age-related permissive milieu or permissive immunologic window. Nevertheless, the connection is important; a young adult with recurrent boils and pneumonia should receive particular attention because the mortality rate of hemorrhagic lung disease is high (Fig. 194.8).

#### Escaping Cell-Mediated Immunity

Among the first lines of skin and mucosal innate defenses are  $\gamma/\delta$  T cells and antigen-presenting Langerhans cells.  $\gamma/\delta$  T cells are not major histocompatibility complex (MHC) restricted and respond to epithelial stress and injury. They promote healing via the production of growth factors and attract neutrophils and T cells via the production of IL-17A,<sup>155</sup> which also upregulates the production of AMPs.<sup>156</sup> Stimulation of

neutrophils by IL-17A decreased the severity of experimental *S. aureus* SSTIs and facilitated *S. aureus* nasal eradication.<sup>156,157</sup> Thus,  $\gamma/\delta$  T cells and the production of IL-17A comprise an important nonspecific first-line defense against invading microbes. Besides, Langerhans cells should phagocytose invading organisms and present surface antigens to boost humoral immunity, thus completing the continuum from innate to acquired host immunity.

However, *S. aureus* is well equipped to counter recognition by phagocytes and migration of neutrophil and lymphocytes, and impede cytokine-mediated cell recruitment and antibody production, including Eap (or Map), which interferes with lymphocyte migration,<sup>130</sup> Spa, Sbi,<sup>134</sup> and Sak (see “Escaping Phagocytosis” earlier). In addition, the most impressive interference of *S. aureus* with cell immunity is the ubiquitous production of SAGs, which trigger massive and nonspecific activation of the T-lymphocyte compartment, resulting in TSS.<sup>2</sup> One consequence of this T-cell distraction is immune paralysis and anergy. SAGs also aggravate atopic dermatitis and psoriasis by promoting local inflammation, serum suffusion, and access to nutrients.<sup>158</sup> SAGs, of which TSST-1 is a paradigm, are discussed in the “Superantigens” section later.

### Producing Biofilm

Biofilm is an ultimate way to settle and escape host defenses. It consists in an extracellular polysaccharidic and proteinaceous meshwork that gathers bacterial communities within a mechanically cohesive scaffold. Biofilm-trapped bacteria cannot be physically phagocytized, a phenomenon referred to as frustrated phagocytosis,<sup>159</sup> and are dormant.<sup>88</sup> As result, they are phenotypically tolerant to antibiotic-induced killing.

Biofilm formation is a major therapeutic problem.<sup>160</sup> It was widely described in CoNS but is also formed by *S. aureus*, especially in the settings of colonization of catheters and biomaterials. Biofilm-producing staphylococci were associated with persistence and virulence in various experimental models, including *Caenorhabditis elegans* and mice with foreign-body infection.<sup>161</sup>

Biofilm formation evolves in three steps, starting with nonspecific adherence of individual cells to the materials, followed by growth and biofilm formation, and ending with detachment of surface bacteria. In CoNS, it is associated with the production of polysaccharide intercellular adhesion (PIA), which consists of  $\beta$ -1,6-glucosamine chains that are *N*-substituted with succinate residues.<sup>162</sup> PIA is synthesized by an operon called *ica* composed of a regulator (*icaR*) and biosynthetic (*icaADBC*) genes.<sup>163</sup>

An *ica* homologue has also been described in *S. aureus*. Its role in colonizing amorphous surfaces might be identical to that shown in CoNS. However, its role in disease initiation is debated.<sup>164</sup> In *S. aureus*, biofilm production relates to a large network of genes including surface-attached and secreted proteins in addition to complex regulatory circuitries.<sup>126</sup> For instance, although biofilm deep-seated bacteria must express adherence molecules, surface bacteria must be prone to detach in order to colonize additional organs. Detachment depends on, among other factors, *agr* expression, which represses expression of adhesins and promotes that of secreted factors including PSMs.<sup>87</sup> In turn, PSMs are involved in remodeling biofilm surfaces and creating channels to feed inner parts of the structure.<sup>165</sup> Thus, *ica* could be a relatively ancestral colonization mechanism that is still present in *S. aureus* but is surpassed by more effective means.

Taken together, the myriad immune evasion strategies collected by *S. aureus* highlight its remarkable adaptation to the animal world and make it a major challenge for host defense-mediated elimination. This explains the as yet unsuccessful attempts to develop an antibacterial vaccine against it, leaving only hope for antitoxin neutralizing vaccines, which will not eradicate the bacterium but might help reduce tissue destruction and disease symptoms.

### Exfoliative Toxins and Staphylococcal Scalded Skin Syndrome

SSSS is a superficial skin disorder that varies from local blistering to impressive generalized scalding (Fig. 194.9). It was originally described by the German physician Baron Gotfried Ritter von Rittershain, who published a series of 297 cases in young children in 1878.<sup>166</sup> Hence, the syndrome is sometimes referred to as Ritter disease. SSSS clusters in



**FIG. 194.9 Staphylococcal scalded skin syndrome.** Blisters are expression of toxin-related (exfoliative toxin A or B) distant disease and usually do not contain microorganisms.

neonates and infants younger than 1 year and rarely in adults. It is typically the result of mucosal or skin colonization (e.g., umbilical cord) with a toxigenic *S. aureus* strain that produces either ETA or ETB, encoded by the *eta* and *etb* genes, respectively. The toxin genes are located either on a phage (*eta*)<sup>8</sup> or on a plasmid (*etb*). Two additional isoforms of SSSS toxins (exfoliative toxins C [ETC] and D [ETD]) were isolated through pathologic observations in animals and with genome screen, but seem not to be involved in humans.<sup>167,168</sup>

A US study estimated the annual incidence of SSSS to be 8 cases per million US children, increasing to 45 cases per million in children younger than 2 years.<sup>169</sup> The crude inpatient mortality was low (0.33%) and similar to that in children without SSSS sharing comparable clinical conditions.<sup>169</sup> Similar figures were reported in France.<sup>4</sup> SSSS is often related to *S. aureus* infections or carriage in close contacts, and may evolve as small epidemics that result from clonally related strains, usually in nurseries. Nasal carriage of the organism may be found among the medical staff, and all caretakers should be screened for this possibility. The proportion of *S. aureus* carrying *eta* or *etb* in overall staphylococcal nasal carriers or clinical isolates is low (<2% of isolates),<sup>170</sup> which explains the rarity of the disease and its clustering in favorable milieus.

The toxins act by a direct effect on the stratum granulosum of the epidermis. Mucosa are never involved. This consideration is important for differential diagnosis with more severe Lyell syndrome, which usually involves mucosa.<sup>3</sup> Lyell syndrome, or toxic epidermal necrolysis, results from cleavage below the dermoeplidermal junction. It is associated with a reaction to more than 100 drugs and sometimes vaccination and has a high fatality rate.

### Molecular Pathogenesis of Staphylococcal Scalded Skin Syndrome

The toxin is released by staphylococci locally, passes through the body, and localizes at the level of the stratum granulosum. The toxin is a glutamate-specific serine protease whose molecular target is desmoglein-1 (Dsg1). Dsg1 is a transmembrane desmosomal glycoprotein that is important to maintenance of interkeratinocyte adhesion.<sup>171</sup> The human skin harbors four Dsg isoforms (Dsg1 to Dsg4) that are localized in various layers of the epidermis, but only Dsg1 is present at the level of the stratum granulosum and is the target of SSSS toxins, which remove its amino-terminal extracellular domain.<sup>172</sup>

An incompletely solved question is why the disease primarily affects children and adults with peculiar skin diseases. One hypothesis is that the toxin targets Dsg1 in the vicinity of the cell membrane ganglioside (GM4), which is present only in the skin of young children or in adults with peculiar skin diseases. This could explain the clustering of SSSS in these particular populations. GM4-like gangliosides are present in the skin of suckling mice and can inhibit the effect of the toxin when coincubated before injection to susceptible animals.<sup>3</sup> The toxin has a serine protease activity, but only after it has reached the skin, which

suggests that a locally induced conformational change is needed for activity.

### Clinical Aspects

The two forms of SSSS are a generalized form and a localized form. In the generalized form, the toxin spreads throughout the body and localizes at the level of the skin, where it produces generalized scalding (see Fig. 194.9). The skin easily detaches by mere rubbing (Nikolsky sign). The blister liquid is clear. Because scalding is the expression of a distantly secreted toxin, the responsible staphylococci are usually not found in the lesions. The disease is self-limited and wanes within 4 to 5 days, which probably parallels the appearance of specific antitoxin immunoglobulins. Indeed, in addition to age-related expression of GM4 or other specific factors in the skin, the presence of antitoxin antibodies in older children and adults also explains the restriction of SSSS to the younger age groups.

The localized form of SSSS is sometimes referred to as bullous impetigo (Fig. 194.10). It results from the local spread of the toxin around a colonized wound in individuals who already bear some immunity against the toxin, as is the case in neonates still benefiting from passive maternal immunity (often around the umbilicus), or in older individuals who are already immunized. The presence of antibodies hinders distant dissemination of the toxin but not local spread around the colonized area. Unlike the generalized form, scalding is localized and the blister liquid often contains bacteria and sometimes white blood cells.

Patients may have general symptoms that include fever and lethargy, especially in the generalized form. Treatment includes general measures such as antiseptic wound dressing and fluid support, specific antibiotic therapy to eradicate the causative agent, and screening and decontamination of caretakers, especially in nurseries. If appropriately handled, the prognosis of SSSS in children is usually good and, as mentioned, the mortality rate far less than 5%.<sup>169</sup> In contrast, the mortality rate can be very high in adults (>50%) and is usually associated with an underlying condition.

As mentioned, the differential diagnosis with Lyell syndrome (toxic epidermal necrolysis) is critical because the etiology, treatment, and prognosis of the diseases are different. In doubtful cases, skin biopsy is useful to provide the definitive answer.

### Superantigens

TSST-1 and staphylococcal enterotoxins (SEs) are the paradigm of a large family of pyrogenic exotoxins called *superantigens*.<sup>2,5,173</sup> SAGs are proteins that do not activate the immune system by means of normal contact between antigen-presenting cells and T lymphocytes. Normally, antigens are taken up by antigen-presenting cells, hydrolyzed, and presented as restricted peptides to cognate T lymphocytes. The peptides are expressed within a groove on the MHC class II receptor on the surface of the antigen-presenting cell. Cognate T cells recognize the peptide–MHC

class II complex by specific contacts with the five variable domains of the  $\alpha$  and  $\beta$  chain of the T-cell receptor (V $\beta$ , D $\beta$ , J $\beta$ , V $\alpha$ , J $\alpha$ ).

SAGs can bypass this highly specific interaction. They attach to an external portion of the V $\beta$  domain from large quantities of lymphocytes and directly wedge them to the MHC class II receptors of antigen-presenting cells. This nonspecific contact activates up to 20% of the total pool of T cells, instead of approximately 1 per 10,000 during normal antigen presentation. The consequence is a massive burst in cytokine release, which drives an overwhelming inflammatory response that results in endotoxin-like shock, including endothelial leakage, hemodynamic shock, multiorgan failure, and possibly death.

*S. aureus* can produce a large number of SAGs. Aside from TSST-1, it can produce at least 15 different enterotoxins (SEs A, B, C<sub>n</sub>, D, E, G, H, I, J, K, L, M, N, O), which by definition are emetic when administered to rodents. However, the nomenclature has become more complicated with the discovery of enterotoxin homologues that did not have emetic properties, and which are now called SEL, for “staphylococcal enterotoxin-like,” followed by specific letterings.<sup>173</sup> Moreover, additional screens have revealed a family of at least 14 proteins based on homologies in the conserved C- and N-terminal domains of SAGs.<sup>174</sup> These proteins do not bind MHC class II molecules but can variously interact with immunoglobulins and complement. They are referred to as SSL for “staphylococcal superantigen-like” and tend to cluster together on staphylococcal pathogenicity islands (discussed in the “Genomics and Mobile Genetic Elements” section later).

Although quite some variation exists in the primary structure of many SAGs, they all share a common architecture, as shown with crystallography. They consist of A and B globular domains, which are made of  $\beta$ -sheet barrels and  $\alpha$ -helices and rejoined by a discrete linking piece. In TSST-1, the region binding to the V $\beta$  chain of the T-cell receptor has been mapped at the A-B hinge region.

A genealogy of SAGs was built on the base of their sequence homologies. The SAGs studied were segregated into five groups.<sup>5</sup> Group I was represented only by TSST-1. Group III contained only staphylococcal SAGs (SEs H, I, K, L, and P), and group IV contained only streptococcal SAGs (SPs E, C, G, and SME Z). On the other hand, groups II and V contained both staphylococcal and streptococcal SAGs. Group II contained staphylococcal SEs B, C, and G and streptococcal SSA and SPE A, and group V contained staphylococcal SEs I, K, L, and P and streptococcal SPs E and H. This underlines the likelihood of horizontal gene transfer between these two genera, a fact that is becoming increasingly apparent with genome comparisons.<sup>2</sup>

### Toxic Shock Syndrome

TSS has been sporadically reported as staphylococcal scarlet fever since 1927.<sup>175</sup> Interest in TSS dramatically increased in the early 1980s, when a number of staphylococcal TSS cases occurred in young women who used high-absorbency tampons during menses.<sup>176</sup> The disease was associated with a toxin called TSST-1 that was secreted locally by toxigenic strains. TSST-1 and other SAGs can cross the mucosal membrane by several means. At the level of mechanical barriers, tight junctions are not uniformly present on mucosal surfaces, and staphylococcal Hla may help further disrupt their surface. At the level of SAGs, a conserved dodecapeptide (YNKKKATVQELD) was shown to mediate transcytosis of the toxin to deeper mucosal layers, promoting contact with immune cells and triggering inflammation—a phenomenon referred to as outside-in signaling mechanism.<sup>5,177</sup> There are two clinical forms of TSS: menstrual TSS and nonmenstrual TSS.

#### Menstrual Toxic Shock Syndrome

Menstrual TSS starts within 2 days of the beginning or the end of menses and is primarily associated with the use of high-absorbency tampons. Clinical signs include high fever, capillary leak syndrome with hypotension and hypoalbuminemia, generalized nonpitting edema, and a morbilliform rash, followed by desquamation after a few days. The toxin is produced locally, and blood culture results are typically negative. The organisms responsible were represented by a single clone in most reported cases.

The disease proceeds by SAG-induced hyperactivation of the immune system (see previous discussion). TSST-1 production is regulated by



**FIG. 194.10 Localized staphylococcal scalded skin syndrome, also called bullous impetigo.** Disease is caused by local production of exfoliative toxins, and bacteria may be found in blister liquid.

*agr* (see Table 194.1). However, its expression requires specific conditions that include (1) an elevated protein level; (2) a relatively neutral pH (6.5–8); (3) an elevated  $\text{PCO}_2$ ; and (4) an elevated  $\text{PO}_2$ .<sup>68</sup> All four conditions are met when menstruation is combined with the use of high-absorbency tampons. The high protein concentration and neutral pH are provided by blood proteins and their buffering capacity. The high  $\text{PCO}_2$  is ensured by the higher than atmospheric  $\text{CO}_2$  content of venous blood. Eventually, the high concentration in  $\text{O}_2$  is introduced into the vaginal anaerobic flora by the high-absorbency tampon. Thus, the  $\text{O}_2$  brought in by the tampon might be the trigger that modifies an otherwise equilibrated ecosystem and stimulates the production of TSST-1 by colonizing staphylococci.

TSST-1-producing *S. aureus* may be found in up to 20% of isolates from both carrier and clinical specimens, and higher in MSSA than in MRSA.<sup>27,170</sup> The fact that TSST-1 expression has special requirements may partially explain the comparatively low prevalence rate of the disease (approximately 1–3 cases per 100,000 patient-years).<sup>2</sup>

#### Nonmenstrual Toxic Shock Syndrome

Nonmenstrual TSS has attracted less attention than menstrual TSS, yet it can occur in any patient. In addition to TSST-1, nonmenstrual TSS can be the result of enterotoxins SEB and SEC, which are *agr* regulated (see Table 194.1). Responsible organisms may colonize virtually any site of the body, including surgical wounds (surgical TSS), lung (influenza-associated TSS), mucosa or skin (recalcitrant desquamative syndrome in patients with acquired immunodeficiency syndrome [AIDS]), contraceptive diaphragms, and dialysis catheters in patients undergoing chronic peritoneal dialysis. The development of general symptoms with high fever and cutaneous rash should suggest the possibility of nonmenstrual TSS in such patients.

A special feature of wound colonization is that the affected tissues often do not appear inflamed. This is believed to result from the toxin itself, which is able to prevent the influx of professional macrophages.

#### Predisposing Factors

In addition to the use of high-absorbency tampons or colonization with a toxicogenic strain, most patients who are TSS susceptible also lack specific antibodies that block the responsible SAg. In one study, antibody titers considered protective against TSST-1 ( $\geq 1:100$ ) were detected in 30% of 2-year-old children and in more than 90% of women and men 25 years of age. However, low or negative titers of anti-TSST-1 antibodies (<5) were found in acute-phase sera from 90.5% of patients with menstrual TSS, and less than 50% had positive titers of anti-TSST-1 antibody that developed during convalescence.<sup>2,178</sup> Hence, some patients remain susceptible to recurrent TSS.

An interesting feature of SAgs is that they primarily trigger a CD<sub>4</sub><sup>+</sup> T-cell response, which privileges a helper T-cell type 1 (Th1) cytokine release response without a significant type 2 (Th2) response. A consequence of the dominant Th1 response is a decreased antibody expression, which could explain the relative lack of antibody response in patients with TSS. An additional explanation for the anergy could be SAg-induced apoptosis of responsive T cells, which could account for the prolonged anergy toward the deleterious toxin.

#### Diagnosis

The diagnosis of TSS is based on a constellation of clinical and laboratory signs as proposed by the Centers for Disease Control and Prevention.<sup>179</sup> Table 194.5 also proposes additional laboratory features, such as isolation of a toxin-producing organism to broaden the diagnostic tools.<sup>180</sup> The criteria of streptococcal TSS, from toxicogenic *S. pyogenes* isolates, are presented for comparison. Although both syndromes are the results of similar kinds of SAgs, they differ in two important aspects. First, in contrast to staphylococcal TSS, streptococcal TSS is almost always associated with the presence of streptococci in deep-seated infections, such as erysipelas or necrotizing fasciitis, which has been referred to as flesh-eating disease. Second, mortality rates are very different in staphylococcal and streptococcal TSS. Mortality rates of menstrual and nonmenstrual (in children) staphylococcal TSS were reportedly <1%.<sup>181,182</sup> In contrast, mortality of streptococcal TSS in children was 28%<sup>182</sup> and up to 45% in adults,<sup>183</sup> especially in cases of necrotizing fasciitis, which

**TABLE 194.5 Diagnostic Criteria for Staphylococcal and Streptococcal Toxic Shock Syndrome**

STAPHYLOCOCCAL TOXIC SHOCK SYNDROME <sup>a</sup>	STREPTOCOCCAL TOXIC SHOCK SYNDROME
Fever	Isolation of group A streptococci from:
Hypotension	Sterile site for definite case
Diffuse macular rash with subsequent desquamation	Nonsterile site for probable case
Three of following organ systems involved:	Hypotension
Liver	Two of the following symptoms:
Blood	Renal dysfunction
Renal	Liver involvement
Mucous membranes	Erythematous macular rash
Gastrointestinal	Coagulopathy
Muscular	Soft tissue necrosis
Central nervous system	Adult respiratory distress syndrome
Negative serologic studies for measles, leptospirosis, and Rocky Mountain spotted fever and negative blood or cerebrospinal fluid cultures for organisms other than <i>Staphylococcus aureus</i>	

<sup>a</sup>Proposed revision of diagnostic criteria for staphylococcal toxic shock syndrome (TSS) includes (1) isolation of *S. aureus* from mucosal or normally sterile site; (2) production of TSS-associated superantigen by isolate; (3) lack of antibody to implicated toxin at time of acute illness; and (4) development of antibody to toxin during convalescence.

Modified from McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. *Annu Rev Microbiol*. 2001;55:77–104.

necessitates aggressive treatment with generous surgical débridement of infected tissues, and sometimes amputation.

#### Therapy and Prevention

Treatment of staphylococcal TSS consists of elimination of the causative agent with antibiotic treatment and appropriate drainage of affected tissues if necessary. Antibiotic regimens should include active drugs such as  $\beta$ -lactams or vancomycin (in case of MRSA) plus protein inhibitors such as clindamycin or linezolid, which block the production of toxins.<sup>184,185</sup> Supportive care that includes intravenous fluid and vasopressors might be necessary. The immunologic gap that allows the toxin to be active in susceptible patients suggests that passive immunotherapy such as intravenous immune globulin (IVIG) could be effective. However, the success of IVIG therapy has been disputed in several recent analyses.<sup>186,187</sup> Because the mortality of menstrual staphylococcal TSS is low, immunotherapy should be considered only for life-threatening cases of streptococcal TSS.

Prevention is aimed at avoiding the use of hyperabsorbent tampons and preventing staphylococcal colonization of wounds and mucosa. In the case of nasal carriage, this is achieved with topical application of antibacterial agents such as mupirocin. In the case of extranasal colonization, additional complete body washing with antiseptics such as chlorhexidine is recommended for at least 1 week (see Table 194.8 later). Control cultures should be taken thereafter.

Active immunization with a TSST-1 vaccine could be a potential alternative. A phase I trial with recombinant TSST-1 demonstrated good tolerance and immunogenicity.<sup>188</sup> Further evaluation is awaited.

#### Enterotoxins and Food Poisoning

*S. aureus* harbors up to 15 enterotoxins, which are defined as SAgs able to produce gastrointestinal symptoms that include vomiting and diarrhea in primate models.<sup>2,5</sup> Although many of these toxins have potential SAg activity, not all of them have a clear role in human disease. As mentioned, SEB and SEC are associated with nonmenstrual TSS. Likewise, SEA is by far the most frequent culprit of food poisoning, whereas SED, SEB, and SEC are less frequently involved.<sup>189</sup>

Foodborne disease is a major public health problem that may account for 6 to 8 million cases per year in the United States. *S. aureus* food poisoning follows ingestion of toxins that have been released into

contaminated food stocks or beverages. The toxins are heat stable and thus are not denatured by cooking. The disease typically starts 2 to 6 hours after ingestion with general malaise, nausea, vomiting, abdominal pain, and diarrhea. No fever occurs, but the symptoms may be distressing enough to justify hospital consultation in approximately 10% of patients. The symptoms spontaneously resolve within 6 to 12 hours, and the prognosis is excellent, except in the case of severe dehydration in young children and elderly patients.

Although the mode of action of SAGs at the level of T lymphocytes is known, their mechanism at the surface of the intestinal mucosa is less clear. They might share transcytosis mechanisms with TSST-1.<sup>5,177</sup>

### Other Implications of Superantigens

Although SAGs can result in dramatic subversion of the host immune system, they are not ultimate bacterial weapons because they affect only a restricted subgroup of patients who do not mount an appropriate blocking antibody response. Many of these staphylococcal genes are physically contiguous, which suggests that they may have arisen by duplication, maybe for the purpose of diversity.<sup>8,190</sup> The versatility of SAGs is further supported by the discovery that one of them (i.e., SHE) develops its SAG activity by binding to the V $\alpha$  rather than the V $\beta$  domains of the T-cell receptor, thus expanding different sets of T-cell lineages than classic SAGs.<sup>191</sup>

The clinical relevance of this multiplicity of toxins is not entirely understood. Toxin genes are dispensable elements that are not needed for growth in rich media and in the absence of competition. Some SAGs (e.g., TSST-1 and SEA, SEB, and SEC) obviously provide a way for the bacterium to escape host immunity. For instance, SAGs have been involved in the etiology of psoriasis and atopic dermatitis,<sup>192</sup> where SAG-induced skin modification could promote bacterial survival. On the other hand, the survival advantage of provoking allergic diseases including rhinitis and asthma<sup>193,194</sup> is less intuitive, except maybe to promote airborne dispersal (see Fig. 194.5). Altogether, the multiplicity of SAGs could enable *S. aureus* to interfere with the immune response of various animal species, thus broadening its host spectrum.

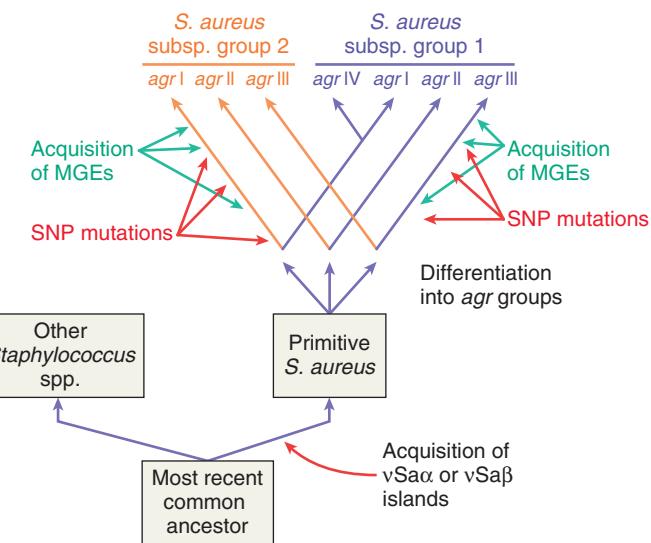
### Genomics and Mobile Genetic Elements

At the time of writing, several thousands of *S. aureus* genome assemblies and annotation reports are available in public databases ([www.ncbi.nlm.nih.gov/genome/genomes/154](http://www.ncbi.nlm.nih.gov/genome/genomes/154)). *S. aureus* genomes are circular and contain approximately 2.8 million base pairs that represent up to 2700 coding sequences, plus structural and regulatory RNAs. They are divided into (1) a core genome, which contains mostly housekeeping genes, is quite conserved along various staphylococcal species, and accounts for about 80% of the whole DNA, and (2) an accessory genome, which carries mobile DNA (MGEs), contains most *S. aureus* pathogenic and drug-resistance features, and may vary among different species and strains.<sup>7,8,10,16–18</sup> In addition, certain elements of the core genome can vary according to lineages—for example, by the presence or absence of core genes that are specific of given clades. Therefore the core genome is sometimes subdivided into the core-stable and core-variable genome.<sup>7,10</sup>

### Comparative Genomics and Evolution

Genome evolution is driven by random point mutations that lead to single nucleotide polymorphism (SNP), larger variations in core genes (e.g., deletions or duplication of repeat regions) that may differ between lineages, and MGEs that include insertion sequences, transposons, viruses, and pathogenicity and genomic islands.<sup>7,8,10,16–18</sup>

Beyond academic interest, understanding the evolution of *S. aureus* may help understand the fundamentals of successful clones and eventually help design strategies to block their spread. Based on 7- and 14-gene MLST analyses,<sup>195,196</sup> an evolutionary scenario was proposed in which a common ancestor of *Staphylococcus* spp. first segregated into non-*S. aureus* and *S. aureus* species (Fig. 194.11). The *S. aureus* branch acquired the genomic islands vSa $\alpha$  and vSa $\beta$  (see later), which are absent from other staphylococci and encode for type I restriction modification systems, and further evolved into two subbranches that gave rise to different STs and CCs. Although still discussed,<sup>196,197</sup> it seems that the global regulator *agr* groups I to IV differentiated early in these two



**FIG. 194.11 Molecular evolution of *Staphylococcus* spp. into species and subspecies.** *Staphylococcus* spp. evolved from a common ancestor into non-*S. aureus* and *S. aureus* species. The *S. aureus* branch distinguishes itself by the early acquisition of the vSa $\alpha$  or vSa $\beta$  genomic islands, which carry the genes for type I restriction modification systems. *agr*-interference groups differentiated shortly after, primarily by mutations. However, the *agr* two-component regulatory system is likely to be very old, because it is also present in non-*S. aureus* species. Sequence types (STs) and clonal clusters (CCs) evolved later, mostly via mutations, and determined two *S. aureus* subspecies (subsp. 1 and subsp. 2). Accordingly, the two subspecies contain different CCs, such as notoriously successful CC30 in subsp. 1 and CC5 in subsp. 2. On the other hand, they may contain similar *agr* interference groups, because these interference groups differentiated earlier, with the exception of group IV. (Modified from Robinson DA, Monk AB, Cooper JE, et al. Evolutionary genetics of the accessory gene regulator (*agr*) locus in *Staphylococcus aureus*. J Bacteriol. 2005;187:8312–8321; and Feng Y, Chen CJ, Su LH, et al. Evolution and pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. FEMS Microbiol Rev. 2008;32:23–37.)

branches, which explains why the four *agr* groups may be found in different downstream CCs (see Fig. 194.11). Eventually, MGEs were acquired later and are dispersed in almost any of the STs or CCs. Yet, a few exceptions to this rule exist. Indeed, the TSST-1 gene and the PVL gene locus are classically associated with *agr* group III, the exfoliatin genes with *agr* group IV, and the vancomycin intermediate-resistance phenotypes with *agr* groups I or II.<sup>7</sup> Whether this is due to peculiarly favorable *agr*-related genetic backgrounds or to favorable contemporary conditions for the extension of specific clones is unclear. Moreover, a functional expression of *agr* is dispensable in certain circumstances, as for instance in biofilms.<sup>87,88,165</sup> Indeed, the association of clinical outcome with *agr* dysfunction and SCCmec type was observed in a study from South Korea, in which MRSA bacteraemia-associated mortality was highest with types II/III, in which *agr* dysfunction is significantly increased.<sup>198</sup>

Using whole-genome sequencing, McAdam and colleagues<sup>199</sup> traced back the evolution of notoriously virulent CC30 to over 100 years ago. In the late 1800s, CC30 first segregated into phage type 80/81 and Southwest Pacific clades, which encoded PVL and produced severe community- and hospital-acquired infections due to penicillin-resistant (but methicillin-susceptible) strains in the mid 1900s. In the mid 1950s, a third hospital-related clade emerged (EMRSA-16), which was devoid of PVL but had acquired a new methicillin-resistance MGE named staphylococcal cassette chromosome *mec* (SCCmec; see later discussion). All three clades gave downstream variants related to further SNPs or MGE acquisitions. Moreover, the CC30 evolution demonstrates acquisition, loss, and reacquisition of PVL and other MGEs, underlying the virulence plasticity of this particular organism. It will be important to understand whether the success of CC30 is due to a peculiar ability to capture useful

MGEs or rather to contemporary environmental conditions that favored clonal expansion (e.g., in the hospital), or perhaps both.

### ***Staphylococcus aureus* Cross-Species Jump Between Humans and Animals**

*S. aureus* is also an animal pathogen that raises particular concerns in livestock and cattle. Human and animal *S. aureus* strains tend to segregate in different lineages.<sup>200</sup> However, cross-species jumps exist and need to be considered. Companion animals and veterinary providers have been shown to share strains.<sup>201</sup> Two outbreaks have been described in Israel involving horses and veterinary personnel.<sup>202,203</sup>

Another example is the swine-related MRSA CC398, which appeared to colonize swine husbandries since the early 2000s.<sup>204</sup> Price and colleagues<sup>205</sup> proposed that CC398 was first transferred from human to swine. Indeed, CC398 is also present in humans and had been devoid of the SCCmec cassette (and thus was methicillin susceptible).<sup>206</sup> The swine CC398 has lost the  $\phi Sa3$   $\beta$ -hemolysin-disrupting prophage, which is present in the human CC398. As mentioned, in human strains prophage  $\phi Sa3$  disrupts the  $\beta$ -hemolysin gene and simultaneously imports virulence factors, including the IEC carrying Sak, CHIPS, and SCIN (discussed under “**Immune Evasion**” earlier).<sup>207</sup> In swine CC398, the loss of prophage  $\phi Sa3$  restores the  $\beta$ -hemolysin gene, which may be important for skin or mucosal colonization in animals.<sup>114,208</sup> The swine CC398 also acquired a new SCCmec cassette that may carry useful determinants for survival in swine husbandries, including antibiotic use. Thus, CC398 was first a human MSSA. It was transferred to swine with the parallel loss of  $\phi Sa3$  and acquisition of a new SCCmec. The loss of prophage  $\phi Sa3$  seems critical in this evolution. This raises the question as to whether reacquisition of prophage  $\phi Sa3$  by swine CC398 could promote its reestablishment in humans, carrying along a new SCCmec cassette. Indeed, cases of invasive human infection with swine-CC398 MRSA are increasingly reported.<sup>209</sup> Likewise, a linear increase of 66% of human CC398 MRSA cases occurred in Denmark from 2004 to 2011; one-third of these patients reported no livestock exposure.<sup>210</sup> Most important, an epidemiologic evaluation in the Republic of Ireland has revealed an elaborate pattern of cases mixing human and animal disease, wherein a few human CC398 MRSA did not carry the  $\phi Sa3$  prophage IEC cluster, thus resembling typical animal strains, whereas some animal CC398 MRSA did carry the  $\phi Sa3$  prophage IEC cluster, thus resembling human strains.<sup>211</sup> In this line, a large study in Iowa found higher rates of *S. aureus* carriage in swine workers, with more than a third carrying livestock-associated strains that did cause infection in humans.<sup>212</sup> This strongly supports the likelihood constant of interspecies passage.

Similar scenarios were described with the ST5 *S. aureus* strain that invades poultry<sup>213</sup> and with the notoriously virulent human CC8 *S. aureus* strain that infects cows.<sup>114,214</sup> As in swine CC398, the bovine version of CC8 first lost the  $\phi Sa3$  prophage and then acquired a new composite SCC element, which is as yet devoid of the methicillin resistance *mecA* gene but carries a new LPXTG surface protein that might be responsible for colonization of the bovine mammary gland. Thus, gene trafficking and genome evolution should be apprehended globally.

### **Pathogenicity and Genomic Islands**

*S. aureus* pathogenicity (SaPI) and genomic islands (vSa) are continuous structures that vary in size from approximately 15 kb to 70 kb and can harbor many virulence or resistance genes. They mostly contain heterologous DNA that indicates exogenous acquisition. A common feature of these elements is that they are bracketed by direct or inverted repeats and carry recombinase genes. The repeats serve as an attachment site (*att*) for integration into homologous regions of the bacterial chromosome. The recombinase, which is often an integrase, catalyzes integration into the chromosome.<sup>215</sup> At least seven SaPIs have been described and are reunified in four groups based on the homology of their integrase genes.<sup>10</sup> SaPI1 and SaPI2 harbor the gene for TSST-1 and are responsible for most cases of TSS. SaPI3 and SaPI4 contain numerous enterotoxin genes. SaPIbov encodes for a bovine version of TSST, and SaPIbap encodes a bovine adherence protein that might play a role in bovine mastitis.

The *S. aureus* chromosome also carries two larger elements called genomic islands (vSa $\alpha$  and vSa $\beta$ ), which were acquired early during staphylococcal speciation (see Fig. 194.11).<sup>8,17,58,190</sup>

vSa $\alpha$  and vSa $\beta$  carry not only restriction modification systems, but also a variety of SEs and other virulence genes and thus have been variously referred to as an enterotoxin gene cluster (*etc*) or virulence gene nursery.<sup>6,173</sup>

Pathogenicity and genomic islands are terminology variations of mobile elements deriving from ancestral prophages, and their mobilization is still dependent on so-called helper prophages.<sup>13</sup> A seminal study showed that *S. aureus* SaPI1 could be mobilized from the bacterial chromosome by  $\phi 80\alpha$  and  $\phi 11$  and transferred into naive recipients thereafter.<sup>216</sup> SaPIs often carry remnants of integrase/excisase (*int/xis*) genes, and  $\lambda$  phage *cl*-like repressor genes that repress the expression of *int/xis* and forbid spontaneous excision of their cognate SaPIs. When induced, helper prophages produce a nonessential protein that blocks the SaPI *cl*-like repressor, thus freeing the expression of *int/xis* genes and promoting SaPI excision. Excised SaPI can replicate and then undergo illegitimate packaging into nascent phage capsids of the helper prophage via *pac* sites. Following phage-induced bacterial lysis, capsids that are illegitimately hijacked by SaPIs can bind to new *S. aureus* recipients and inject their SaPI-DNA cargo, a process called generalized transduction. The delivered SaPI DNA then undergoes Campbell-like site-specific integration into the bacterial chromosome using its *att* site and its own integrase.<sup>13,217</sup> Such phage mobilization was observed with other pathogenicity islands (e.g., SaPI2) and is likely to be a general mechanism of gene transfer for these large elements. Excision and transfer of such elements are triggered by stresses such as exposure to ultraviolet light and certain antibiotics *in vitro*.<sup>216–218</sup> They are also likely to be promoted by antibiotics in the clinical setting as well.

### **Resistance Island Staphylococcal Cassette Chromosome *mec***

MRSA contains one resistance island called *staphylococcal cassette chromosome (SCC) mec*, wherein *mec* is the genetic element that confers resistance to methicillin.<sup>219</sup> SCCmec is an exogenous piece of DNA that may vary between 15 and 60 kb. It inserts at the 5' end of the *orfX* gene at a conserved 15-base-pair chromosomal attachment site (*attB*) that recombines with an homologous site on the cassette (*attS*), generating two flanking direct repeats (*attL* and *attR*) after insertion. The SCCmec critical genes are the recombinases *ccrA/ccbB* and *ccrC*, which can mediate mobilization of the whole element, and the *mecA* gene, which mediates  $\beta$ -lactam resistance. The rest of SCCmec contains various additional determinants and is referred to as “J” for junkyard.<sup>220</sup>

*mecA* encodes for penicillin-binding protein 2A (PBP2A), which has a low affinity for methicillin and most other  $\beta$ -lactam drugs and confers intrinsic resistance of MRSA to almost all  $\beta$ -lactams (see “**Mechanism of Methicillin Resistance**”).<sup>221</sup> The *mecA* gene is preceded or not by the *mecRI* and *mecI* regulator determinants, which are homologues of the *blaRI* and *blaI* regulators of penicillinase (*bla*) genes. *mecRI* (and *blaRI*) encodes for a membrane receptor, and *mecI* (and *blaI*) encodes for a gene repressor. In the presence of penicillin, the extracellular portion of the membrane *mecRI* (*blaRI*) receptor triggers an autocatalytic cleavage of its intracytoplasmic portion. The liberated intracytoplasmic peptide acts as a metalloprotease, which further cleaves the *mecI* (*blaI*) repressor, thus derepressing gene expression.<sup>222</sup> The *mecA* gene is bracketed by one or two copies of IS431, which is believed to serve as a gene collector and might promote the local insertion of additional determinants, such as antibiotic resistance genes.<sup>220</sup>

Several types of SCCmec have evolved from more generalist SCC cassettes, which are structures able to integrate a variety of genes and are functional equivalents to integrons in gram-negative bacteria. Up to 12 SCCmec types have been described according to a consensus classification system.<sup>223,224</sup> The classification is based on the *mecA* complex, which encompasses the *mecA* gene and its surrounding regulatory genes, and the *ccr* complex, which includes the *ccrA/ccbB* and *ccrC* genes.<sup>223</sup> The *ccrA/ccbB* and *ccrC* genes do not come together on the same SCCmec. *ccrA/ccbB* are cotranscribed and come in four different alleles, whereas *ccrC* has only one allele. Up to now there are nine types of SCCmec that carry various combinations of *ccrA* and *ccbB* and three types that carry one of two different *ccrC* alleles, including one in type V and VIII and a newer one in type XII.<sup>224</sup>

*SCCmec* types mirror major original MRSA clones. Types I, II, and III were shown to belong to HCA-MRSA. They harbor multiple resistance determinants, they have relatively large sizes (35–60 kb), and they are therefore difficult to mobilize. Types IV, V, and VI were associated with CA-MRSA.<sup>225,226</sup> They are much smaller (about 15 kb) than their hospital congeners and do not carry multiple antibiotic-resistance genes. However, they appear to be associated with other elements in the same bacterium, including prophage-related PVL and multiple SE genes.<sup>8</sup> One particularly successful clone of CA-MRSA (clone USA300 of CC8 lineage) has also acquired a so-called arginine catabolic mobile element (ACME) inserted downstream of the *SCCmec* cassette.<sup>227</sup> ACME was acquired from *S. epidermidis* and confers survival advantages in acidic and maybe other environments. It improved survival and fitness of USA300 in a rabbit model of bacteremia.<sup>228</sup> Together these elements may render the organism particularly fit and virulent.

Whereas HCA-MRSA types are clonal and carry large and difficult-to-mobilize *SCCmec* cassettes, CA-MRSA types carry small *SCCmec* cassettes, which are more prone to mobilization<sup>220</sup> and less clonal than HCA-MRSA, at least in Europe, where the USA300 clone is rather sporadic.<sup>229,230</sup> CA-MRSA did not arise from HCA-MRSA that permeated the community, but emerged independently by acquiring *SCCmec* most likely from CoNS donors.

Recently, a new *SCCmec* carrying a *mecA* variant gene (*mecA<sub>LGA251</sub>*) with only 70% homology to *mecA* was missed by molecular probing and incidentally identified through methicillin-resistance phenotyping.<sup>48</sup> This variant, renamed *mecC*,<sup>47</sup> was mainly found in livestock-associated MRSA (e.g., CC130, CC1943, and ST425). Although few *mecC*-positive MRSA strains could be traced back to the mid-1970s,<sup>231</sup> they have clearly been increasing since 2003, representing almost 3% of all MRSA isolates recovered in Denmark in 2011.<sup>231</sup> This adds to the need to closely follow staphylococcal cross-species jumps between humans and animals.

Beyond epidemiologic implications, the use of molecular diagnostics insensitive to non-*mecA* methicillin resistance should be considered, and a newer generation of the Cepheid Xpert (new Xpert MRSA Gen 3; Cepheid, Sunnyvale, CA) PCR assay includes *mecC* as a target.<sup>212</sup> The therapeutic implications of *mecC* have not been reported, but this variant PBP has a higher affinity for penicillins than for cephalosporins, and *in vitro*, *mecC* isolates are susceptible to the addition of clavulanate to penicillin.<sup>232</sup> This is reminiscent of the greater affinity of old penicillin G and amoxicillin for PBP2A, and the fact that amoxicillin-clavulanate combinations successfully cured experimental infections due to MRSA, with clavulanate being required to block secreted penicillinase.<sup>233</sup> Moreover, expression of methicillin-resistance by *mecC*-positive MRSA appears to be temperature dependent. MICs of flucloxacillin (an analogue of methicillin) were much lower when tested at 37°C than at 30°C. As a result, flucloxacillin successfully cured experimental endocarditis due to *mecC*-positive MRSA, whereas it is known to fail against *mecA*-positive MRSA.<sup>234</sup>

## ANTIBIOTIC RESISTANCE

*S. aureus* has developed resistance to virtually all antibiotic classes available for clinical use. These encompass cell wall inhibitors such as β-lactams and glycopeptides; ribosomal inhibitors that include macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>), pleuromutilins, aminoglycosides, tetracyclines, fusidic acid, and the new oxazolidinones; the RNA polymerase inhibitor rifampin; the DNA gyrase-blocking quinolones; the antimetabolite TMP-SMX and iclarpim; and the newer lipopeptides and lipoglycopeptides.<sup>235–238</sup> FDA-approved versions of such drugs and the main resistance mechanisms of *S. aureus* are summarized in Table 194.6. Some are discussed subsequently.

### β-Lactams

β-Lactams inhibit bacterial growth by interfering with cell wall assembly. They bind to the active site of a series of membrane-bound enzymes responsible for inserting the peptidoglycan precursors into the nascent wall (Fig. 194.12).<sup>239</sup> Certain enzymes are bifunctional and carry both transglycosidase and transpeptidase activity. Transpeptidation takes place at the D-ala-D-ala terminal of the precursor. It hydrolyzes the covalent bond between the penultimate and the terminal D-ala and

then transfers the penultimate D-ala to a free NH<sub>2</sub> terminal (the ε-NH<sub>2</sub> of lysine) of neighboring peptidoglycan stem peptides. The terminal D-ala is released, and a new stem peptide cross-link is created (see Fig. 194.12A).

Penicillin and other β-lactams are steric analogues of the cell wall D-ala-D-ala terminal of the precursors. They are mechanism-based inhibitors that compete with D-ala-D-ala binding to the active site of the membrane-bound transpeptidase, which they block irreversibly—hence the term *penicillin-binding protein* (PBP) coined for these enzymes.

### Resistance to Penicillin

The most common resistance mechanism of *S. aureus* to β-lactams is penicillinase, which is encoded by the *bla* gene usually carried on a plasmid. The gene is inducible and preceded by the *blaRI* and *blaI* regulatory determinants (see “Resistance Island Staphylococcal Cassette Chromosome *mec*”). Penicillinase is a secreted enzyme that hydrolyzes penicillin and other penicillinase-susceptible compounds into inactive penicilloic acid. Penicillinase-producing *S. aureus* emerged rapidly after penicillin was introduced as a therapeutic agent in the mid 1940s. It is now prevalent both in the hospital and in the community, where it represents close to 80% of the isolates.<sup>240,241</sup>

The minimal inhibitory concentration (MIC) of penicillin G for fully susceptible *S. aureus* is approximately 0.01 mg/L. In contrast, the MIC of penicillinase-stable drugs such as nafcillin, oxacillin, flucloxacillin, or cephalosporins is 10-fold greater. Thus, penicillin G remains the best choice against penicillin-susceptible staphylococci. However, major concerns remain in the United States about recommended detection of penicillin susceptibility and resistance, because currently recommended susceptibility assays may miss more than 35% of β-lactamase-producing *S. aureus*.<sup>242,243</sup> Moreover, the use of a single β-lactam, such as oxacillin or cefoxitin, to predict susceptibility of *S. aureus* to other β-lactams may have limitations. For example, an analysis of *S. aureus* MICs found that oxacillin was a better predictor than cefoxitin for susceptibility to ceftriaxone, ceftaroline, and cefazolin.<sup>244</sup>

### Methicillin-Resistant *Staphylococcus aureus*

The first penicillinase-stable β-lactams such as cephalosporins and semisynthetic methicillin and nafcillin became available in the late 1950s. Ironically, MRSA was first described at about the same time.<sup>245</sup> The prevalence of MRSA progressively increased thereafter. One survey of the National Nosocomial Infections Surveillance System (NNIS) found that the hospital prevalence of MRSA increased from 2.1% in 1975 to 35% in 1991.<sup>246</sup> It went up to 60% in certain centers in the United States,<sup>52</sup> and more than 70% in a report from Shanghai,<sup>247</sup> but great geographic variations exist worldwide. Several reports have indicated a declining trend in the prevalence of MRSA over the last decade, both in Europe and in North America.<sup>53,248–250</sup> Although this decline most probably results from hospital hygiene measures, the exact reason may be more intricate, such as a better fitness of MSSA replacing MRSA in the absence of antibiotic pressure.<sup>251</sup> This was elegantly demonstrated in competition experiments showing that antibiotic concentrations that were several orders of magnitude below the MIC of susceptible strains favored the growth of MRSA over MSSA, whereas MSSA overgrew MRSA in the total absence of antibiotics.<sup>252</sup> Such low antibiotic concentrations are found in sewages of urban environments<sup>253</sup> and are likely to be decreasing with antibiotic consumption control, thus favoring MSSA over MRSA resurgence.

### Health Care–Associated Versus Community-Acquired Methicillin-Resistant *Staphylococcus aureus*

Although originally confined to the hospital environment, MRSA has emerged as a community-associated infection over the past 2 decades.<sup>25,153,254</sup> CA-MRSA is different from HCA-MRSA from both epidemiologic and molecular points of view. Case-definition studies showed that HCA-MRSA and CA-MRSA represented different organisms that produced different clinical syndromes.<sup>255,256</sup> HCA-MRSA was associated with risk factors that included recent hospitalization or surgery, residence in a nursing home, or presence of an indwelling catheter or device. It produced mostly hospital-related pneumonia and



**TABLE 194.6 *Staphylococcus aureus* Resistance Mechanisms to Major Classes of Antibiotics**

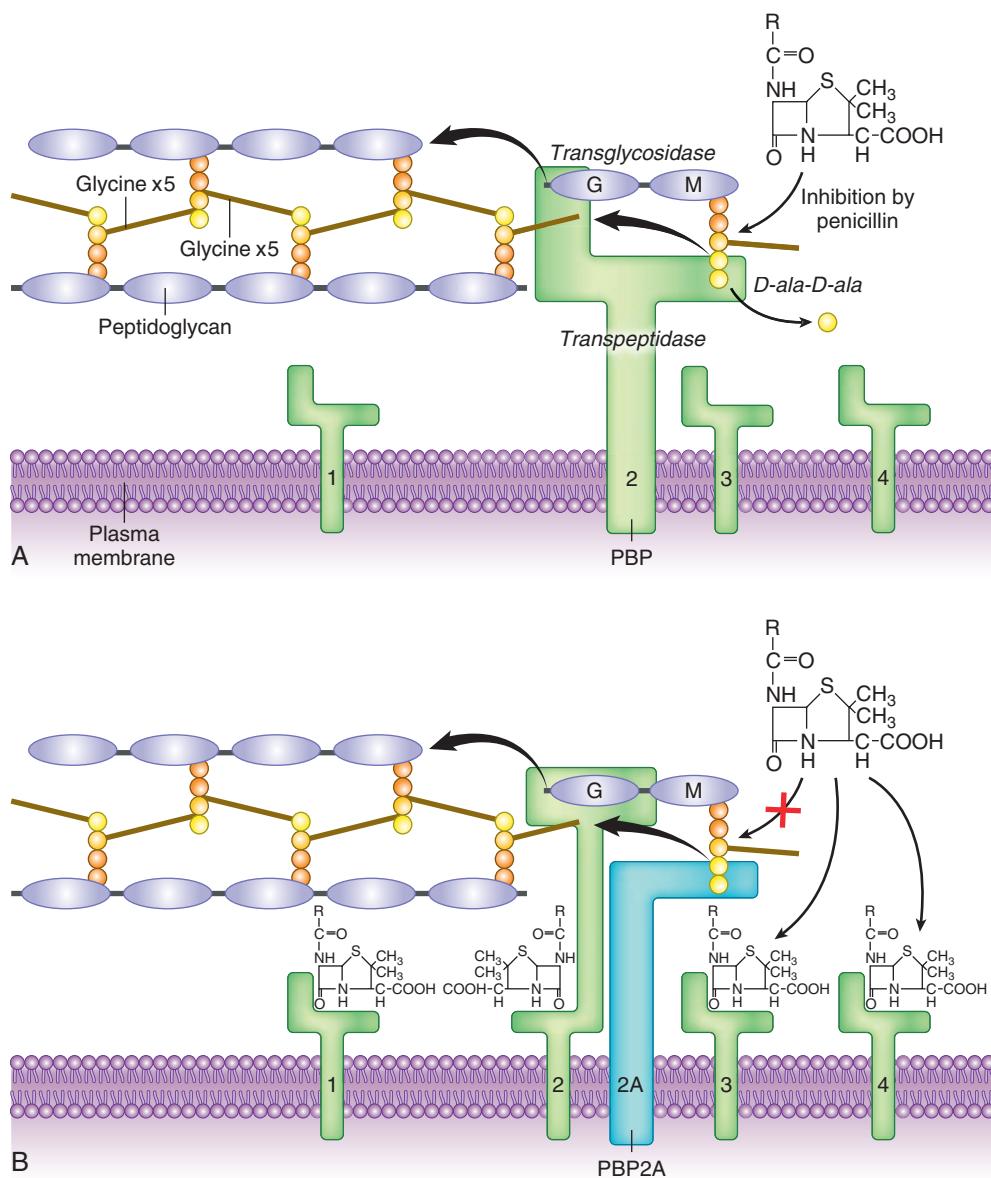
ANTIMICROBIAL AGENTS	TARGET MODIFICATION	DRUG INACTIVATION	DECREASED ACCUMULATION	NATURE	RESISTANCE GENE		
					ORIGIN	LOCATION <sup>a</sup>	
β-Lactams Penicillinase-S	Yes	Yes	No	Penicillinase PBP2A <sup>b</sup> (PBP2C in some livestock animals)	Acquired Acquired	Plasmid SCCmec (chromosome)	
	Penicillinase-R	No	No	PBP2A <sup>b</sup> (PBP2C in some livestock animals)	Acquired	SCCmec (chromosome)	
Glycopeptides Intermediate resistance Full resistance	Yes	No	No	Mutations in wall-building genes vanA and vanH	Intrinsic	Chromosome	
	Yes	No	No	Mutations in genes involved in wall-building and membrane charges (mprF)	Acquired	SCCmec (chromosome)	
Lipoglycopeptides Daptomycin	Yes	No	No		Intrinsic	Chromosome	
Macrolide-lincosamide-streptogramin B Macrolides	Yes	No	Yes	erm	Acquired	Plasmid or chromosome	
				msrA	Acquired	Plasmid	
				erm	Acquired	Plasmid or chromosome	
				linA	Acquired	Plasmid or chromosome	
Lincosamide <sup>c</sup>	Yes	Yes	No	erm	Acquired	Plasmid or chromosome	
				vgb (rare)	Acquired	Plasmid or chromosome	
Streptogramin B <sup>c</sup>	Yes	Yes	Yes	msrA (rare)	Acquired	Plasmid or chromosome	
				vat, vatA (rare)	Acquired	Plasmid or chromosome	
Streptogramin A	No	Yes	Yes	vga, vgb (rare); combinations of above (rare)	Acquired	Plasmid or chromosome	
Quinupristin-dalfopristin	Yes	Yes	Yes				
Linezolid	Yes	No	No	Mutation in 23S rRNA gene cfr	Intrinsic Acquired	Chromosome Plasmid	
Tetracyclines	Yes	No	Yes	tet(M), tet(O) tet(K), tet(L)	Acquired Acquired	Plasmid or chromosome Plasmid or chromosome	
Gentamicin	No	Yes	Yes	aac(6')-aph(2') Respiratory chain mutants	Acquired	Plasmid or chromosome Chromosome	
Chloramphenicol	No	Yes	No	cat	Acquired	Plasmid or chromosome	
Fusidic acid	Yes	No	Yes	fusA mutation pUB101	Intrinsic Acquired	Chromosome Plasmid	
Rifampin	Yes	No	No	rpoB mutation	Intrinsic	Chromosome	
Fluoroquinolones	Yes	No	Yes	gyrA and gyrase norA	Intrinsic Intrinsic	Chromosome Chromosome	
Trimethoprim	Yes	No	No	dfrA mutation dfrA	Intrinsic Acquired	Chromosome Plasmid or chromosome (acts by mutation or overproduction)	
Sulfamethoxazole	Yes	No	No	dpsA	Intrinsic Acquired	Chromosome Plasmid (probable) (acts by mutation or overproduction)	

<sup>a</sup>SCCmec, Staphylococcal chromosomal cassette *mec* (see text for details).<sup>b</sup>PBP2A, Penicillin-binding protein 2A; PBP2C, penicillin-binding protein 2C.<sup>c</sup>erm gene must be induced or constitutively expressed to confer resistance to lincosamides and streptogramins B. Only macrolides are good inducers. Lincosamides and streptogramins do not induce resistance but are inactive against constitutively MLS<sub>B</sub>-resistant strains.

aac(6')-aph(2') Bifunctional aminoglycoside acetyl-transferase and phosphor-transferase determinant, present on transposons Tn4001; cat, chloramphenicol acetyl-transferase; cfr (*chloramphenicol-florfenicol resistance*), 23S rRNA methyltransferase; dfrA, dihydrofolate reductase gene; dpsA, dihydropteroate synthase; erm, erythromycin-resistance methylase, mainly ermA (chromosome, transposons Tn554) and ermC (plasmid); fusA, gene encoding elongation factor G (EF-G); gyrA and gyrase, genes encoding for the DNA topoisomerase and gyrase, respectively; linA, lincosamide nucleotidyl transferase; mprF (*muropeptide resistance factor*), lysylphosphatidylglycerol synthase; msrA, macrolide-streptogramin resistance, ABC-transporter; norA, gene encoding for staphylococcal efflux pump; pUB101, plasmid encoding penicillin-resistance (penicillinase), cadmium-resistance, and a protein (Far1) conferring impermeability to fusidic acid; rpoB, gene encoding for β subunit of RNA polymerase; tet(M) and tet(O), responsible for ribosomal-modification and protection; tet(K) and tet(L), responsible for active efflux of tetracyclines; vanA and vanH, vancomycin resistance A and H genes (see text for details); vat and vatA, acetyl transferase genes; vga and vgb, streptogramin A efflux gene, ABC-transporter; vgb, virginiamycin hydrolysis.

bacteremia. In contrast, CA-MRSA was not associated with any risk factors and produced primarily SSTIs (often furunculosis), and sometimes rapidly fatal necrotizing pneumonia. It was also described as being responsible for necrotizing fasciitis and bone and joint infections.<sup>257,258</sup> In addition, whereas HCA-MRSA was multiresistant and highly clonal, CA-MRSA was pauciresistant<sup>259,260</sup> and seemingly more polyclonal, at

least in Europe and Australia.<sup>229,260</sup> Indeed, CA-MRSA has been highly clonal in North America, with a first clone (USA400 and ST1) prevailing until approximately 2000. Since then, it has been replaced with the very successful and highly prevalent clone USA300 (ST8),<sup>153</sup> accounting for 61% of all MRSA isolates in the United States in one recent surveillance survey.<sup>261</sup> Most interesting, Strauss and colleagues<sup>262</sup> traced USA300 back



**FIG. 194.12 Peptidoglycan assembly in wild-type *Staphylococcus aureus* and in methicillin-resistant *S. aureus* (MRSA).** (A) Cell wall precursors consist of units of disaccharides-peptides where disaccharides (*N*-acetylglucosamine-*N*-acetylmuramic acid) are linked to pentapeptides (L-alanine-D-glutamate-L-lysine-D-alanine-D-alanine) at the *N*-acetylmuramate residue. After membrane translocation, precursors are handled by membrane penicillin-binding proteins (PBPs). High-molecular-weight PBPs are bifunctional enzymes that perform both a transglycosidase step, linking incoming *N*-acetylglucosamine (G) to muramic acid (M) in nascent wall, and a transpeptidase step, linking penultimate D-alanine to glycine acceptor in nascent wall. In *S. aureus*, lysine in position 3 of stem peptide is almost always decorated with pentaglycine side chain (orange bars). Penicillin is a mechanism-based inhibitor of the transpeptidase domain of PBPs. (B) MRSA carries an additional PBP called PBP2A, which has very low affinity for most available β-lactam drugs. Therefore, when β-lactams are present, they block normal PBPs but not PBP2A. PBP2A has only a transpeptidase domain and must "hijack" the transglycosidase domain of normal PBP2 to be active. (From de Lencastre H, Wu SW, Pinho MG, et al. Antibiotic resistance as a stress response: complete sequencing of a larger number of chromosomal loci in *Staphylococcus aureus* strain COL that impact on the expression of resistance to methicillin. *Microb Drug Resist*. 1999;5:163–175.)

to Europe—not to Australia, as formerly thought—where it became prevalent as PVL-negative MSSA in the mid-1800s. It then spread through Europe and invaded the United States in the early 1900s, where it acquired PVL-containing prophage φSA2 in the late 1930s, followed by the ACME locus and then several introductions of SCCmec after the 1960s. Since then, it has spread transcontinentally and intercontinentally to South America and Africa but as yet has not become prevalent in Europe.

As mentioned, CA-MRSA is associated with SCCmec type IV (and type V and VI in a few cases) and almost always carries PVL.<sup>153,259,263</sup> PVL is epidemiologically associated with SSTI and necrotizing pneumonia, but its specific role in disease has been controversial because, among other considerations, experimental results were opposite in rabbit

versus mouse experimental models.<sup>228,264,265</sup> This issue was solved by two more recent studies. The first one demonstrated that PVL toxicity was host specific.<sup>266</sup> PVL appeared to bind to and lyse neutrophils via the C5a receptor and was effective against human and rabbit versions of C5a, but not against mouse and dog C5a, thus explaining the rabbit and mouse differences. The second demonstrated that PVL was toxic in humanized SCID mice, but not in wild-type mice.<sup>267</sup> These are important findings because they provide a rationale for treating PVL-producing staphylococcal infection with toxin-blocking antimicrobial combinations containing, for instance, clindamycin or linezolid,<sup>268</sup> or maybe passive immunotherapy.<sup>269</sup>

Thus, HCA-MRSA and CA-MRSA are not alike. Practically, MRSA in patients at risk is likely to be of the multiresistant hospital type,

whereas MRSA in patients without risk factors is likely to more susceptible to antibiotics but more invasive.

### Mechanism of Methicillin Resistance

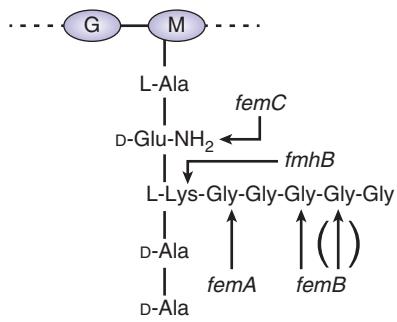
The main mechanism of methicillin resistance is not mediated by penicillinase but by the newly acquired PBP2A, encoded by *meca*.<sup>221</sup> The few staphylococci that express borderline methicillin resistance from the overexpression of penicillinase are usually not considered as clinically relevant, although awareness of their possible implication in poor therapeutic outcome is resurging.<sup>270</sup> Because of its low  $\beta$ -lactam affinity, PBP2A can take over the cell wall assembly when normal staphylococcal PBPs are blocked by  $\beta$ -lactams (see Fig. 194.12B).<sup>271</sup> However, although this confers high intrinsic resistance to virtually all  $\beta$ -lactams, PBP2A has a special requirement for particular cell wall precursors. These must contain a pentaglycine decorating side chain attached to the position 3 L-lysine of their stem peptide and other specificities, such as an amidated D-glutamine in position 2 of the peptide (Fig. 194.13).

Providing this adequate substrate to PBP2A requires the functionality of several additional genes implicated in the normal wall building machinery, including 14 or more accessory determinants.<sup>272</sup> Some of them (*femABC* and *fmbH*) are responsible for adding the glycine side-chain residues critical for the PBP2A function.<sup>273</sup> Any alteration in these elements decreases the expression of methicillin resistance in spite of the fact that PBP2A is present.

Another fragility of PBP2A is that it carries only a transpeptidase domain and misses a transglycosidase activity (see Fig. 194.12B). Thus, for successful assembly of the peptidoglycan, PBP2A needs the assistance of the transglycosidase domain of normal staphylococcal PBP, namely PBP2.<sup>274</sup> This is a salient example of protein cooperation in antibiotic resistance but also represents the Achilles' heel of the system. Because most  $\beta$ -lactams can readily block the normal staphylococcal PBPs, further drug development needs only to target additional PBP2A to be effective. Both experimental work and recent crystallographic evidence indicate that such an approach is feasible.<sup>275</sup> Successful treatment of experimental endocarditis from MRSA was achieved with an array of older and newer  $\beta$ -lactams with good PBP2A affinity.<sup>233,276</sup> This approach is driving the development of new anti-MRSA compounds,<sup>277</sup> which recently generated some novel molecules of the cephalosporin (e.g., ceftobiprole and ceftaroline) and carbapenem classes.<sup>278,279</sup>

### Glycopeptides

As a general rule, current *glycopeptides* (e.g., vancomycin) are less bactericidal than  $\beta$ -lactams against MSSA. Therefore they should not be used as first-line treatment against  $\beta$ -lactam-susceptible organisms.<sup>280</sup> However, vancomycin is still a gold standard against severe MRSA infections, recently enriched by daptomycin, novel anti-MRSA  $\beta$ -lactams (which are discussed later), and combinations of vancomycin or daptomycin plus  $\beta$ -lactams to take advantage of the so-called "seesaw effect" (also discussed in "Daptomycin" and "Alternatives Treatments" later).



**FIG. 194.13 Peptidoglycan precursor required for wall assembly by PBP2A.** To be functional, penicillin-binding protein 2A (PBP2A) requires that the cell provide fully decorated precursors, containing both pentaglycine side chain and amidated glutamine. Inactivation of *femB*, *femA*, and *fmbH* genes blocks addition of pentaglycines and thus decreases expression of methicillin resistance even though PBP2A is present in bacterial membrane. Inactivation of *femC* has a similar effect. (Modified from Berger-Bächi B. Expression of resistance to methicillin. Trends Microbiol. 1994;2:389–393.)

Two types of resistance to glycopeptides were reported in clinical isolates of *S. aureus*, namely "intermediate" and "high-level" resistance. According to the 2012 guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS])<sup>281</sup> the vancomycin MIC breakpoints for *S. aureus* are as follows:  $\leq 2$  mg/L for susceptible isolates; 4 to 8 mg/L for intermediate-resistant isolates; and  $\geq 16$  mg/L for high-level-resistant isolates. Both resistance phenotypes result from different mechanisms and may be of different clinical and epidemiologic relevance.

### Therapeutic Monitoring of Vancomycin in Adult Patients

Although CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; [http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/)) agree on the  $\leq 2$  mg/L susceptibility breakpoint, several reports and meta-analyses have indicated that infections due to *S. aureus* with vancomycin MICs  $> 1$  mg/L but  $\leq 2$  mg/L (referred to as high MICs) result in a greater mortality than infections due to *S. aureus* with vancomycin MICs  $\leq 1$  mg/L (referred to as low MICs).<sup>282,283</sup> Therefore, special attention was given to pharmacokinetic and pharmacodynamic parameters that could help predict treatment efficacy.<sup>284</sup> A consensus review proposed the following guidelines for vancomycin treatment and monitoring in severe infection, based on twice-daily administration of the drug:

From the therapeutic point of view:

1. The trough concentration of vancomycin in the serum is the best indicator of efficacy and prevention of resistance selection.
2. Trough levels of vancomycin should be between 15 and 20 mg/L.
3. Obtaining peak levels is not indicated.
4. Drug dosages should be adapted to the patient's body weight and renal function. A loading dose of 25 to 30 mg/kg IV must be considered, followed by 15 to 20 mg/kg IV q12h depending on the renal function and drug dosage monitoring.

From the toxicity point of view:

1. Unlike original preparations of vancomycin (in the late 1950s), ototoxicity is no longer an issue if vancomycin is given in monotherapy.
2. Nephrotoxicity at high doses in monotherapy has been reported.
3. Vancomycin-induced nephrotoxicity should be considered if two to three consecutive measurements of renal function are abnormal during therapy.
4. Peak concentration of vancomycin does not seem to be a good correlate of toxicity.

Applying appropriate vancomycin dosage might explain the results of a more recent study reporting no difference in the mortality rate of patients infected with low or high vancomycin MIC *S. aureus*.<sup>285</sup>

### Intermediate Resistance to Glycopeptides

Intermediate-resistant *S. aureus* isolates were originally described in Japan and in the United States<sup>286,287</sup> but are ubiquitous. The first isolate,<sup>286</sup> called Mu50, was recovered from a 4-month-old child with MRSA sternal wound infection after cardiac surgery. The infection did not respond to vancomycin treatment. The organism had an MIC of vancomycin of 8 mg/L, as detected with standard broth dilution methods. At that time, the CLSI defined staphylococci for which the MIC of vancomycin was 4 mg/L or less as susceptible, for which the MIC was 8 to 16 mg/L as intermediate, and for which the MIC was 32 mg/L or more as resistant.<sup>29</sup> Therefore, the Mu50 isolate was defined as a vancomycin (or glycopeptide)-intermediate *S. aureus* (GISA).

The same author reported that a second *S. aureus* isolate, called Mu3, was responsible for vancomycin treatment failure in an adult patient with pneumonia.<sup>288</sup> Although the vancomycin MIC for this isolate was 4 mg/L, formally considered as susceptible at the time, Mu3 contained GISA subpopulations ( $\leq 10^{-6}$  colony-forming units) that grew in the presence of 5 to 9 mg/L of vancomycin and were not detected with standard drug-susceptibility testing. The term *heteroresistant GISA* (hGISA) was coined to define the Mu3 phenotype. Since then, a number of cases of GISA and hGISA have been described worldwide and were associated with vancomycin treatment failures both in animal experiments and in human cases.<sup>289</sup> As mentioned earlier, CLSI and EUCAST breakpoints now classify isolates with a vancomycin MIC of

4 and 8 mg/L as GISA. Currently the term “heteroresistant” is applied to isolates with a vancomycin MIC of 2 µg/mL or less but which harbor resistant colonies (see Chapter 30).

Intermediate glycopeptide resistance arises from pleiotropic chromosomal mutations that affect the structure of the wall peptidoglycan. In susceptible strains, glycopeptides inhibit cell wall assembly by binding to the D-ala-D-ala terminal of cell wall precursors and block both transpeptidation and transglycosylation. GISA harbors a thickened cell wall that contains an increased number of free non-cross-linked D-ala-D-ala terminals. This increased amount of free D-ala-D-ala is believed to act as a lure that traps glycopeptide molecules before they reach their target.<sup>290</sup>

Genomic analyses indicate that mutations in two-component sensing systems are involved, including *vraSR* (for vancomycin resistance-associated sensor/regulator),<sup>291</sup> *graSR* (for glycopeptide resistance-associated regulator),<sup>292,293</sup> and *walKR*.<sup>294</sup> *vraSR* is a cell wall stress response regulator that affects the expression of up to 100 genes, including cell-wall building enzymes such as PBP2 and MurZ.<sup>291</sup> *vraSR* dysfunction affects peptidoglycan sturdiness, which is compensated for by wall thickening. *graSR* affects the expression of up to 200 genes including the *dlt* operon and muropeptide resistance factor *mprF*. As mentioned in the “**Immune Evasion**” section, the *dlt* operon and *mprF* are involved in cell surface charge modulation by means of alanylation of teichoic acids and lysine decoration of membrane phospholipids, respectively.<sup>142</sup> This explains the phenomenon of GISA cross-resistance with AMPs and daptomycin, the antibacterial activities of which depend on membrane charges (see also discussion later).<sup>295</sup> *walKR* is a wall metabolism-associated regulon that is highly conserved in low GC gram-positive bacteria and senses bacterial wall changes—for instance, in response to antibiotic exposure—in order to adapt cell wall metabolism.<sup>296</sup> *walKR* mutations affect both vancomycin and daptomycin resistance by means of *mprF* deregulation, among other processes.<sup>294,296</sup>

Experimental work indicates that like SCVs, GISA strains preexist and are selected during therapy.<sup>297</sup> However, their low level of resistance and sometimes-heterogeneous phenotype make them hard to detect in the laboratory.<sup>29</sup> Pending the development of efficient automated systems, a convenient low-technology dual-antibiotic Etest strip assay containing vancomycin and teicoplanin performed remarkably well. It detected GISA and hGISA with a high sensitivity (95%) and specificity (94%), which was almost identical to much more cumbersome population analysis, which is not performed in routine laboratory testing.<sup>298</sup>

## Full Resistance to Glycopeptides

Full vancomycin resistance (MIC ≥16 mg/L for *S. aureus*) has been known for more than two decades in *Enterococcus* spp.<sup>299</sup> In these organisms, glycopeptide resistance results from the acquisition of either Tn1546 or Tn1547, two transposons that encode for a series of genes that modify the D-ala-D-ala terminal of the bacterial peptidoglycan precursor, the very target of glycopeptide compounds, to D-ala-D-lactate. The modified D-ala-D-lactate-containing precursor has a low affinity for glycopeptides and therefore confers resistance. Tn1546, which encodes the so-called VanA resistance phenotype, could be transferred to *S. aureus* experimentally.<sup>300</sup> Thus, the recent emergence of fully vancomycin-resistant *S. aureus* (VRSA) that expresses the VanA phenotype among human clinical isolates is not astonishing. Only 14 cases were described in the United States until 2015,<sup>301</sup> but these organisms must be taken seriously. First, most patients had evidence of previous MRSA and enterococcal infection, but not all had received vancomycin. Thus, transfer of the transposon may occur by more generalized triggering effects, perhaps involving unrelated antibiotics. Second, the VRSA phenotype may be missed with routine automated antibiotic susceptibility testing<sup>302</sup> and could be more prevalent than observed. Third, new cases were described in hospitals in the Middle East.<sup>303</sup> Fourth, bloodstream isolates of both methicillin-susceptible and methicillin-resistant, but fully vancomycin-resistant, have been described in Brazil. The conjugative plasmid carrying the *vanA* cluster has been identified, and in vitro transfer demonstrated.<sup>304,305</sup> Finally, a few isolates of VanA-positive MRSA were recovered in a screen of river surface water in Turkey.<sup>306</sup> Taken together, this information indicates that VRSA is pending and warrants constant attention in the diagnostic laboratory.

## Daptomycin

Daptomycin is a relatively new lipopeptide that was approved in the United States and elsewhere for *S. aureus*-complicated SSTI, bacteremia, and right-sided infective endocarditis.<sup>236</sup> It is increasingly used as a replacement for vancomycin against MRSA. Daptomycin is an amphiphilic molecule that requires calcium to solubilize as octamer-micelles in liquid phases.<sup>307</sup> Because of its large size, it cannot traverse the outer membrane of gram-negative bacteria, which are naturally resistant to the drug. In gram-positive organisms, it diffuses through the peptidoglycan toward the plasma membrane, where the calcium ions disperse and leave the lipid moiety of daptomycin to interact with the plasma membrane and destabilize its electric potential.<sup>308</sup> Daptomycin is highly bactericidal, but its activity is dose dependent and the dosage of the drug must be large enough to ensure supra-MIC tissue levels. Standard recommendations are 4 to 6 mg/kg IV q24h, but 8 or even 10 mg/kg q24h have been used in severe infections without notable side effects.<sup>309–311</sup> Prospective observational studies and large retrospective series indicate that daptomycin may also be considered for use against left-sided infective endocarditis due to MRSA,<sup>310,312</sup> but approval by official agencies is still pending. Daptomycin should not be used against airway-acquired pneumonia because it is inactivated by alveolar surfactant.<sup>313</sup>

Mutants with decreased daptomycin susceptibility (MIC >4 mg/L) were recovered both in the laboratory and in clinical samples.<sup>314,315</sup> As mentioned in the “**Intermediate Resistance to Glycopeptides**” section earlier, daptomycin resistance is mediated by mutations in or deregulation of *mprF* (via *vraSR* or *graSR*), which increases the charge of staphylococcal plasma membrane, and is often associated with intermediate vancomycin resistance.<sup>315,316</sup> This cross-resistance raises caution in antibiotic use.

Interesting to note, however, is the fact that daptomycin-resistant mutants have a decreased fitness that may hamper their virulence and dissemination.<sup>317</sup> Moreover, like intermediate vancomycin resistance, daptomycin resistance is associated with the seesaw effect, characterized by a resensitization to β-lactams related to a general perturbation of the cell-cell building machinery and an alteration of PBP expression.<sup>318,319</sup> Taking advantage of the seesaw effect opens ways to alternative antibiotic combination strategies.

## Protein Synthesis Inhibitors

The MLS<sub>B</sub> antibiotics and the oxazolidinone linezolid are discussed in this section. The tetracycline derivatives glycylcycline tigecycline and omadacycline are addressed in the subsequent section “**Alternative Treatments**.”

## MLS<sub>B</sub> Antibiotics

MLS<sub>B</sub> antibiotics comprise separate classes of molecules (i.e., macrolides, ketolides, lincosamides, and streptogramin B) that all bind to the bacterial 50S ribosomal subunit and block protein synthesis. Resistance proceeds by any of the three classic mechanisms: modification of the bacterial drug target, modification-inactivation of the drug itself, and decreasing intracellular accumulation of the drug.

Ribosome modification and drug efflux are the most frequent resistance mechanisms in *S. aureus*.<sup>320</sup> Ribosome modification is mediated by the *erm* gene (for erythromycin methylase), which encodes a methylase that adds one or two methyl groups to the 23S rRNA of the 50S ribosomal subunit. This inflicts a steric alteration that greatly decreases the affinity of the drug for its target. The *erm* determinants belong to a family of methylase genes preferentially located on mobile elements such as transposons (e.g., Tn554 and *ermA*) or plasmids (e.g., pE194 and *ermC*). An additional sophistication in *S. aureus* is that the expression of *erm* may be inducible or constitutive.<sup>320</sup> In the case of the inducible form, the *erm* product is synthesized only in the presence of inducing drugs. Thus, the bacterium does not spend worthless metabolic energy in the absence of antibiotic pressure. Among MLS<sub>B</sub> drugs, only macrolides are good *erm* inducers. However, once induced, the gene product confers cross-resistance to the other members of the group, including the newer ketolides, lincosamides, and streptogramin B, but not streptogramin A. Moreover, mutations that result in constitutive *erm* expression, and hence, global MLS<sub>B</sub> resistance, occur at high frequency ( $10^{-7}$  to  $10^{-8}$ ). Therefore, lincosamides (e.g., clindamycin) should be used with great caution against *erm*-inducible isolates (i.e., resistant to erythromycin

but susceptible to lincosamides and streptogramin B) because the drug might select for constitutive MLS<sub>B</sub> mutants, which are resistant to the whole group of compounds.<sup>321</sup>

Newer drugs of the ketolide subfamily, including telithromycin and solithromycin, have a greater ribosomal affinity, are poor *erm* inducers, and have lower MICs than erythromycin for *S. aureus*.<sup>322</sup> However, they are still affected by constitutive expression of *erm* and thus are not useful against these types of organisms, which include the majority of HCA-MRSA isolates.<sup>322–324</sup>

In the laboratory, the MLS<sub>B</sub> resistance phenotype is detected with the disk diffusion D-test in which erythromycin and clindamycin disks are placed at a distance on a plate inoculated with bacteria and the diffusion of erythromycin toward the clindamycin disk induces clindamycin resistance. As a result, the zone of inhibition around the clindamycin disk takes a D shape.<sup>321</sup> In contrast, constitutive MLS<sub>B</sub> resistance yields no inhibition zone at all around the clindamycin.

### Drug Efflux

Active macrolide efflux has been reported in both streptococci and staphylococci.<sup>325,326</sup> In *S. pyogenes* and *Streptococcus pneumoniae*, efflux is mediated by the *mefA* and *mefE* genes, respectively, which are members of the major facilitator transporter and export only macrolides (M-resistance phenotype). *S. aureus* and CoNS may contain *msrA*, which belongs to the complex ABC-transporter (adenosine triphosphate [ATP]-binding cassette) set of genes<sup>325</sup> and confers resistance to both macrolides and streptogramin B (MS-resistance phenotype). In contrast to major facilitators, ABC transporters use ATP hydrolysis as a source of energy for active efflux. The *msrA* complex is located on a plasmid and is frequently observed in MLS<sub>B</sub>-resistant CoNS. It can be transferred into *S. aureus*,<sup>325</sup> but its clinical relevance for MLS<sub>B</sub> resistance is unclear because it is rarely detected in clinical isolates.<sup>327</sup> Of note, lincosamides (e.g., clindamycin) are not subject to efflux by these pumps.

Constitutive MLS<sub>B</sub> resistance, associated or not with drug efflux, is extremely frequent (>90%) in HCA-MRSA.<sup>324</sup> Therefore, use of MLS<sub>B</sub> drugs should never be considered against such organisms. The only exception is the quinupristin-dalfopristin combination (streptogramin B and A; see “Alternative Treatments”). In contrast, only 5% of CA-MRSA isolates are reported as clindamycin resistant and mostly are of the inducible phenotype.<sup>153,328</sup> Thus, clindamycin remains a therapeutic option against CA-MRSA.

### Oxazolidinones

The oxazolidinone linezolid prevents initiation of protein synthesis by binding to the 23S rRNA of the 50S ribosomal subunit, near its interface with the 30S subunit. It is active only against gram-positive bacteria and is essentially bacteriostatic. It is approved in the United States for complicated SSTI and nosocomial pneumonia from susceptible organisms, including MRSA. Although originally controversial, linezolid was shown to be superior to vancomycin against MRSA nosocomial pneumonia (daptomycin cannot be used in pulmonary infections) in a randomized double-blind study.<sup>329</sup> A systematic review on infective endocarditis from multiresistant bacteria, including 18 MRSA and vancomycin-intermediate *S. aureus* (VISA) isolates, reported a success rate of about 60%, which suggests that compassionate use of linezolid might be an option in such complicated situations.<sup>330</sup> Moreover, evidence-based reviews indicate that it is equal to vancomycin in a number of clinical situations<sup>331</sup> including osteomyelitis in children.<sup>332</sup>

One asset of linezolid is that it can be administered orally and thus is useful for outpatient therapy. Another is that, like clindamycin, it inhibits the secretion of TSST-1 and other toxins and should be considered against toxin-associated infections, including CA-MRSA hemorrhagic pneumonia.<sup>268,333</sup> On the other hand, linezolid is not suitable for long-term (>28 days) therapy because prolonged treatment may be associated with thrombocytopenia, sometimes peripheral or optic neuropathy, and lactic acidosis.<sup>334</sup>

Linezolid resistance has been reported episodically in clinical settings. It is primarily the result of mutations in the 23S rRNA gene.<sup>335</sup> Because staphylococci harbor six to seven copies of rRNA genes, mutation in only one of them does not yield high-level resistance at once. MIC increments are progressive, and MICs of such mutants are usually 4 to

8 mg/L compared with a baseline of 2 mg/L. However, plasmid-mediated high-level resistance was also detected in clinical isolates of *S. aureus* and of *S. epidermidis* (MIC, 8 and >257 mg/L, respectively).<sup>336</sup> The resistance gene (*cfr*) encodes a 23S rRNA methylase that confers cross-resistance to other drugs that bind at the same site, including chloramphenicol, lincosamides (i.e., clindamycin), and streptogramin A.<sup>337</sup> Plasmid-born *cfr* was recently reported in several isolates from livestock in Europe and from human patients in the United States and Ireland.<sup>338,339</sup> Nevertheless, the overall rate of linezolid resistance in *S. aureus* clinical isolates remains very low (<1%) even after more than 15 years of use.<sup>340,341</sup>

The newer semisynthetic tedizolid derivative, approved both in the United States and in Europe for SSTI, has 2x to 8x lower MICs than parent linezolid for MRSA and is not affected by *cfr*-mediated resistance.<sup>342</sup> It is noteworthy, however, that the improved in vitro efficacy is partially offset by a greater binding to plasma proteins—that is, 85% as compared with 30% for linezolid.<sup>343</sup> In two phase III trials of acute skin and skin structure bacterial infections, tedizolid 200 mg once daily for 6 days compared favorably with linezolid 600 mg twice daily for 10 days.<sup>344</sup>

### Quinolones

Quinolones are an important class in the antiinfective armamentarium. They originated in the 1960s as a byproduct from the synthesis of antimalarial quinines. Fluorinated derivatives such as ciprofloxacin, norfloxacin, and ofloxacin appeared in the 1980s. They had low MICs (on the order of 0.01 mg/L) for most gram-negative pathogens. However, the MIC for gram-positive bacteria was relatively high (0.25–2 mg/L for *Staphylococcus* spp. and *Streptococcus* spp.)<sup>345</sup> and close to therapeutic concentrations in the serum of humans (2 mg/L for peak concentration of ciprofloxacin). Use of these borderline active drugs against MRSA facilitated the selection for resistant derivatives. The prevalence of quinolone resistance in HCA-MRSA has been around 90% for a long time and is close to 40% in CA-MRSA,<sup>229,346</sup> which makes older and newer quinolones mostly inappropriate against MRSA.

### Mechanisms of Resistance

Known quinolone-resistance mechanisms result from chromosomal mutations (see Table 194.6). Plasmid-mediated resistance has been described in gram-negative pathogens and is associated with the *qnr* gene, which protects the quinolone targets.<sup>347</sup> A *qnr*-like gene has been described in *Enterococcus faecalis* and could confer resistance to *S. aureus*.<sup>348</sup> However, such a mechanism was not yet described in clinical isolates.

Quinolone resistance proceeds by two types of mechanisms, including overexpression of the efflux pump NorA<sup>349</sup> and structural mutations in the quinolone targets topoisomerase IV (*grlA* and *grlB*) and gyrase (*gyrA*, *gyrB*) genes.<sup>350</sup> Resistance is acquired stepwise. A first *grlA* mutation, which occurs at frequencies of 10<sup>-7</sup> to 10<sup>-8</sup>, produces a moderate increase in MIC (e.g., 0.5–2 mg/L of ciprofloxacin) that is still considered susceptible (<4 mg/L). However, this first mutation paves the way to a second mutation in the *gyrA* gene, which, combined with the *grlA* mutation, results in high-level resistance. Because the initial *grlA* mutation jeopardizes the efficacy of quinolones, it is critical to avoid selecting it at first, by ensuring appropriate drug levels in the blood and tissues.

Older quinolones readily select for such alterations, yielding highly resistant organisms after only a few serial exposures to the drug.<sup>351</sup> Quinolones with improved anti-gram-positive activity (levofloxacin, moxifloxacin, gatifloxacin, garenoxacin) are less selective. However, they may still select for higher resistance levels in bacteria that already acquired a first degree (*grlA* mutants) of ciprofloxacin resistance (MIC, 2–8 mg/L).

The newer agent delafloxacin might be more promising. Its modified chemical structure ensures dual binding to gyrase and topoisomerase IV, thus resulting in 10 to 100 times lower MICs than previous quinolones, including against *grlA* mutants, in addition to insensitivity to NorA-mediated efflux.<sup>352</sup> At the time of writing, delafloxacin is still investigational.<sup>237</sup>

Pharmacokinetic and pharmacodynamic criteria help predict quinolone efficacy and risk for resistance. Efficacy was predicted by peak drug-level/MIC ratios of 8 or more and ratios of area under the concentration-time curve/MIC of 100 or more.<sup>353</sup> With regard to resistance prevention, in vitro and in vivo experiments have suggested

that drug dosage might be adjusted on so-called mutation prevention concentration, which is two to four times higher than the MIC, rather than on MIC.<sup>354</sup> However, the clinical relevance of mutation prevention concentrations has not yet been assessed.

### Alternative Treatments

Treatment of infections from multiresistant HCA-MRSA may be problematic. The activity of all available drugs must be tested against the isolate to establish whether some could still be used. Evaluation of the severity of the disease is also important because not all drugs are equally appropriate in serious conditions.

### Alternatives Against Non-Life-Threatening Infections

Superficial and non-life-threatening infections may respond to a variety of drugs, including TMP-SMX, to which HCA-MRSA and CA-MRSA are often susceptible, combined or not with other substances such as rifampin. Other alternatives include the agents discussed in the following sections.

#### Tigecycline

A modified version of minocycline from the tetracycline family of molecules, tigecycline is almost universally active against gram-negative and gram-positive pathogens, with the notorious exception of *Pseudomonas aeruginosa* and a few other gram-negative organisms that can extrude the drug via efflux pumps.<sup>355</sup> Tigecycline overcomes current *S. aureus* tetracycline-resistance mechanisms, including ribosome protection and active efflux, and thus is effective against all tetracycline-resistant isolates. It is approved in the United States and Europe for the treatment of complicated SSTI. However, it is strictly bacteriostatic, and no trials on *S. aureus*-specific severe infections have been reported. It is still uniformly efficacious against MRSA in recent antimicrobial surveys.<sup>356</sup> The drug must be administered intravenously. Increased mortality in the aggregated clinical trials is also a concern.<sup>357</sup> The drug should not be used in severe situations.

#### Aminomethylcyclines

This new class of minocycline derivatives, structurally related to tigecycline, includes two recently approved drugs, omadacycline (see Chapter 26) and eravacycline. Both have promising in vitro activity against *S. aureus*, both methicillin-susceptible and methicillin-resistant isolates, but insufficient clinical data to estimate their use.

#### Quinupristin-Dalfopristin

Quinupristin-dalfopristin combines a streptogramin B (quinupristin) and a streptogramin A (dalfopristin).<sup>358</sup> It is active against both MLS<sub>B</sub>-susceptible and MLS<sub>B</sub>-resistant staphylococci. It is highly bactericidal against MLS<sub>B</sub>-susceptible isolates, but tends to be less bactericidal in the case of constitutive MLS<sub>B</sub> resistance, which is practically always the case with HCA-MRSA. Standard dosage is 7.5 mg/kg IV q12h, but larger doses (7.5 mg/kg q8h) have been suggested against constitutive MLSB resistant strains. Experimental data indicate that combining quinupristin-dalfopristin with a β-lactam increases its activity against MRSA, even though the β-lactam is inactive on its own.<sup>359</sup> One limitation of quinupristin-dalfopristin is its venotoxicity, which makes it necessary to administer the drug through a central catheter. Arthralgia and myalgia are significant side effects.

#### Lipoglycopeptides

Semisynthetic derivatives of glycopeptides, lipoglycopeptides carry modifications of specific functional groups. Three lipoglycopeptides are currently approved for SSTIs in the United States: telavancin, dalbavancin, and oritavancin.<sup>237,360,361</sup> Like vancomycin, they bind to the D-alα-D-alα terminal of peptidoglycan precursors, thus inhibiting both transpeptidation and transglycosylation. In addition, the presence of lipophilic pharmacophores allows the molecules to interact with the plasma membrane, which leads to dispersion of the membrane potential and rapid bacterial killing. Their lipophilic nature confers high binding ( $\geq 90\%$ ) to plasma proteins and thus prolongs their plasma half-life.<sup>362</sup> For instance, dalbavancin can be given only once a week.

All three compounds are active against antibiotic-susceptible and antibiotic-resistant (including vancomycin) gram-positive pathogens and showed efficacy in various animal infection models. In a double-blind complicated SSTI trial, dalbavancin given once weekly for 2 weeks (1 g at day 1 and 0.5 g at day 8, IV) was highly effective (success rate, >90%) and comparable to linezolid given twice a day (600 mg IV q12h; may be later switched to oral therapy) for the same length of time.<sup>363</sup> Oritavancin given 1200 mg IV once for acute SSTI, including MRSA infections, gave comparable results to vancomycin 1 g (or 15 to 20 mg/kg) IV q12h for 7 to 10 days.<sup>364</sup> The high cost of dalbavancin and oritavancin appears to factor in the cost of intravenous vancomycin administration.

Of note, however, high binding to plasma protein may hamper diffusion of the drug inside therapeutic sanctuaries such as abscesses or infected vegetations. This limitation was demonstrated years ago with teicoplanin<sup>365</sup> and might have been responsible for recently described treatment failure of dalbavancin in right-sided *S. aureus* endocarditis.<sup>366</sup> Data are lacking regarding use of this drug family against severe *S. aureus* infections.

### Alternatives Against Severe Infections

There is a notorious lack of good alternatives for use in deep-seated or life-threatening infection. Vancomycin is one of the first choices in such situations. However, poor response to vancomycin may occur in a substantial proportion of patients because the drug is poorly bactericidal and selects for resistant mutants.<sup>282,283,297</sup>

#### Combination Therapy

Combining vancomycin with an aminoglycoside increases in vitro bactericidal activity. However, the clinical benefit of adding aminoglycosides to vancomycin is not demonstrated, and both kidney and ototoxicity are a matter of concern.<sup>367</sup>

An interesting alternative is to take advantage of the vancomycin-β-lactam or vancomycin-daptomycin seesaw effects,<sup>318</sup> as discussed in the “Daptomycin” section earlier. The fact that resistance to methicillin or daptomycin and resistance to vancomycin are mutually exclusive has yielded several successful therapeutic attempts.<sup>368</sup> An open-label study in MRSA bacteremia demonstrated that combining vancomycin with flucloxacillin shortened the duration of bacteremia to 1.94 days, as compared with 3 days with vancomycin.<sup>369</sup> Moreover, the so-called CAMERA2 trial is now studying vancomycin or daptomycin combinations with antistaphylococcal β-lactams (flucloxacillin, cloxacillin, or cefazolin) on a larger scale.<sup>370</sup>

In addition, there is a growing interest in combining fosfomycin plus β-lactams (e.g., imipenem) as a rescue therapy for complicated MRSA bacteremia or endocarditis.<sup>371</sup> This combination is still being evaluated.

#### β-Lactams With Improved Penicillin-Binding Protein 2A Affinity

Improving the affinity of β-lactams for MRSA-specific PBP2A has been the purpose of intensive research.<sup>278</sup> Work on the structure of PBP2A and potential blocking of PBP2A-β-lactams indicates that the active site of the enzyme is closed in the resting state and thus difficult to reach with the drug. However, when the enzyme is exposed to cell wall precursors or β-lactams with appropriate pharmacophores, allosteric interactions at other portions of PBP2A trigger opening of the active site, providing access to the precursors or to the blocking drug.<sup>372</sup> The success of this interaction probably depends on the hydrophobic pentaglycine side chain of the precursor (see Figs. 194.12 and 194.13) and thus on the presence of hydrophobic pharmacophores on the β-lactam molecule.<sup>278</sup> This poses solubility problems, which were solved by delivering the compound as a prodrug, as in ceftobiprole medocaril.

Ceftaroline is the first of these agents approved for use in the United States for complicated skin and skin structure infections and nonstaphylococcal community-acquired pneumonia (CAP). It has activity against MSSA and MRSA, with MICs for MRSA of 0.5 to 2 mg/L, and 0.12 to 0.25 mg/L for MSSA. The “AWARE” susceptibility survey, which gathered 21,046 *S. aureus* isolates from 42 US centers from 2010 to 2016, showed persistent (>97%) susceptibility to ceftaroline.<sup>341</sup> Ceftaroline was equivalent to ceftriaxone in MSSA pneumonia; MRSA pneumonia was not

systematically studied. Ceftaroline has been examined in parallel studies of complicated skin and skin structure *S. aureus* infections in comparison with vancomycin-aztreonam. The ceftaroline-vancomycin population microbiologic profiles consisted of 73.4% and 84% *S. aureus* infection (34.3% and 32% MRSA; 39.9% and 50.9% MSSA), respectively. Clinical cure rates were 95.1% and 91.4%, respectively, with microbiologic and adverse event outcomes that were similar to those in the comparator.<sup>373,374</sup> The clinical use of the drug is compromised by the lack of a comparative study in major MRSA diseases, such as bacteremia, endocarditis, and osteomyelitis. Multiple reports have focused on its activity in vitro against isolates nonsusceptible to vancomycin and daptomycin, and its clinical use in combination with other agents for the treatment of severe MRSA infections such as bacteremia and endocarditis, which does not answer the question of whether ceftaroline alone would be an effective treatment.<sup>341,374–376</sup> Case series of salvage treatment in refractory MRSA bacteremia, pneumonia, and endocarditis (including prosthetic valve infection) have been reported and suggest that the drug may be an effective option, alone or in combination.<sup>319,377–380</sup>

## Development of Nonantibiotic and Vaccine Strategies

A number of nonantibiotic strategies are being investigated to circumvent *S. aureus* antibiotic resistance. These encompass novel AMPs, virulence modulators, antibiotic resensitization, immunomodulation, bacteriophages (phages), phage endolysins (lysins), and vaccines.

### Antimicrobial Peptides

AMPs include primarily cathelicidin-like peptides purified from various animal or insect venoms, and synthetic derivatives of them.<sup>381,382</sup> AMPs are positively charged amphiphilic peptides. It was recently shown that artificially increasing their charges increased their bactericidal activity as well.<sup>383</sup> On the other hand, *S. aureus* resists the detrimental effect of AMPs by increasing the charge of its own cell wall or plasma membrane via the *dlt* operon and *mprF*, which act as repelling factors (discussed in “[Immune Evasion](#)” earlier).<sup>142</sup> Whether increasing the AMP charges will upset the bacterial resistance mechanism is not clear. Moreover, AMPs still have relatively high MICs for *S. aureus*.<sup>383</sup>

### Virulence Modulation

Virulence modulators include primarily inhibitors of SortA, which block the anchoring of *S. aureus* surface adhesins<sup>96,98</sup>; iron-capturing determinants, which also promote infection<sup>97,99,102,384</sup>; and inhibitors of *agr*, which block the secretion of toxins.<sup>61,64</sup> Although the blocking of surface protein and toxin secretion is conceptually sound, none of these methods reached total protection in animal models.<sup>385,386</sup>

Because *S. aureus* virulence results from the intertwining of both surface-attached and secreted factors, one should ideally administer the two kinds of inhibitors simultaneously to prevent or dampen infection severity. However, although promising, this method will probably not offset the need to eradicate the microorganisms from the infection site.

### Immunomodulation

Reports on the production of IL-17A by  $\gamma/\delta$  T cells and the benefit of IL-17A in stimulating innate immunity and impeding skin and nasal colonization by *S. aureus* are quite promising<sup>156,157</sup> (see also “[Escaping Cell-Mediated Immunity](#)” earlier). Another strategy consists of sensitizing *S. aureus* cells to complement and phagocytes, referred to as “lysibodies”<sup>387</sup>; this is described in the “[Phage Lysins](#)” section later. Whether prescribing IL-17A or lysibodies will be beneficial in humans remains to be determined.

### Antibiotic Resensitization

Statins have pleiotropic effects that alter membrane compartmentalization in both eukaryotes and prokaryotes<sup>388</sup> and were associated with improved clinical outcomes in infected patients.<sup>389,390</sup> In the case of MRSA, it was recently shown that antibiotic resensitization was due to altered PBP2A positioning.<sup>391</sup> The authors demonstrated that PBP2A positioning in the cell membrane depended on the dynamics of “functional membrane micro-domains” (FMMs), which are the bacterial equivalent of membrane rafts in eukaryotic cells. FMMs depend on the interaction of

membrane lipids with the scaffold protein “flotillin,” with which statins interfere. Thus, statins alter both eukaryotes and prokaryotes,<sup>388</sup> in which they may restore susceptibility to antibiotics—that is, to methicillin and its derivatives in the case of MRSA. This remarkable antibacterial benefit of statins, associated with additional in vitro<sup>392</sup> and clinical observations,<sup>389,390</sup> warrants further scrutiny.

Another antibiotic sensitization study addressed the eradication of intracellular *S. aureus* persistence. It showed that conjugating antibody and antibiotics in a system in which antibiotics became activated only in the phagolysosomes was superior to vancomycin in eliminating intracellular *S. aureus* and treating bacteremia in mice.<sup>393</sup> However, once again, the limitation may be the antiphagocytic strategies of *S. aureus*.

### Phage Lysins

Phage lysins and phage therapy are coming close to clinical applicability.<sup>394,395</sup> Phages produce peptidoglycan hydrolases (lysins) to burst the host bacteria and release their progeny at the end of their replication cycle. Lysins can be purified and used to lyse gram-positive bacteria very effectively from the outside. Gram-negative bacteria are protected from outside-in lysis diffusion by their outer membrane and are thus naturally resistant to lysis therapy. Several anti-*S. aureus* phage lysins were produced, and demonstrated potent in vitro and in vivo activity in animal models<sup>396–398</sup>; however, few successful cases of treatment of refractory *S. aureus* skin infections in humans have been reported.<sup>399</sup> Lysins are not yet available for routine clinical use, but two prospective clinical trials are underway in *S. aureus* dermatitis<sup>400</sup> and BSIs (<https://clinicaltrials.gov/ct2/show/NCT03163446?term=cf-301&rank=1>).

An interesting development of lysins in the field of immunomodulation is via so-called lysibodies.<sup>387</sup> It consists of fusing a lysis peptidoglycan-binding domain to an IgG Fc fragment. The lysis peptidoglycan-binding domain of the construct binds to the conserved peptidoglycan and exposes its Fc portion at the bacterial surface. The Fc portion attracts complement and professional phagocytes and promoted bacterial clearance both in vitro and in a mouse sepsis model.<sup>387</sup> It remains to be determined whether *S. aureus* will be able to circumvent lysibodies via their immune escape armamentarium (discussed in “[Immune Evasion](#)” earlier).

### Phage Therapy

The strategy of phage therapy is generating growing interest.<sup>395</sup> Phages have been used against bacteria since the early 1920s, but were abandoned in Western countries after the introduction of antibiotics. In contrast, phages were continuously developed in the countries of the former Soviet Union, particularly at the Eliava Institute in Tbilisi, Georgia.<sup>401</sup> Phages are very rapidly bactericidal and can synergize with antibiotics.<sup>402</sup> Aside from ample experience from the Eastern countries, no reported phase III comparative clinical trials on phage therapy have been performed, with the exception of the multicenter European Phagoburn study on burn wounds infected with *P. aeruginosa* (<http://www.phagoburn.eu>), the results of which are still preliminary. On the other hand, there are recent case reports on successful phage therapy of *S. aureus* osteomyelitis and diabetic ulcers,<sup>401,403</sup> and at least one ongoing phase I study using anti-*S. aureus* phages in chronic rhinosinusitis is planned (<https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368275>). Phage therapy is promising, but it still requires careful comparative assessment.

### Vaccines

Vaccination is a very important area of research both in human and in veterinary medicine. However, considering the arsenal of immune evasion strategies of *S. aureus* (discussed in “[Immune Evasion](#)” earlier), this issue is an immense challenge. Vaccines pursue one of three aims: blocking the effect of toxins, blocking the functional surface adhesins or other relevant proteins, or stimulating phagocytosis. Experimental vaccines have been developed by a variety of means, including DNA vaccines, and against constituents as diverse as the capsule or specific surface determinants, including PBP2A and adhesins. Most of these vaccines conferred some protection in experimental models, and in one trial a conjugated capsular vaccine conferred promising but transient

protection in patients on hemodialysis.<sup>404</sup> A trial using a vaccine targeted on the IsdB iron-binding LPXTG-bound protein to prevent deep sternal wound infections did not provide protection and paradoxically increased the mortality rate in *S. aureus*-infected patients.<sup>405</sup>

One critical point regarding extrapolation of results in mouse models to human is that mouse thrombocytes do not carry a platelet immunoglobulin Fc receptor similar to FcγRIIA in human thrombocytes.<sup>406</sup> As discussed in the “[Contribution of Coagulation](#)” section, *S. aureus* activates platelets and hijacks them to colonize tissues with inflamed or injured endothelia. Hence, antibodies directed to the *S. aureus* surface expose Fc fragments that in turn activate platelets via FcγRIIA (in humans) instead of binding complement and attracting leukocytes, and help channel staphylococcal aggregates to injured tissues. In contrast, this is not expected to occur in the case of neutralizing antibodies that are directed against soluble toxins, which might be a better option. These opposed effects of surface-directed versus toxin-directed antibodies are convincingly supported by studies examining vaccination against experimental PVL-related pneumonia.<sup>407</sup>

As yet, no approved antistaphylococcal vaccine is available for clinical use, but there is one pending phase I/II study targeting leukocidins (<https://clinicaltrials.gov/ct2/show/results/NCT01011335?term=staphylococcus+aureus&draw=5&rank=43>), and hope still exists regarding the targeting of mixtures of *S. aureus* surface-bound and secreted determinants.<sup>408</sup>

## **CLINICAL ASPECTS AND EPIDEMIOLOGY**

Over the last 2 decades, the increase in medical procedures such as major operations, chronic dialysis, organ transplantation, and cancer chemotherapy has profoundly modified the epidemiology of *S. aureus* infections worldwide. With the recent rise in invasive procedures, *S. aureus* has become a leading cause of nosocomial and health care–related infections.<sup>409,410</sup> Along with the pressure toward shorter lengths of stay in acute-care settings, increasing numbers of such health care–related *S. aureus* infections are now diagnosed in subacute or long-term care facilities.<sup>411,412</sup>

*S. aureus* invasive infections consistently result in mortality rates as high as 20% to 30%,<sup>413–415</sup> especially when associated with antibiotic resistance (i.e., methicillin resistance)<sup>411</sup> or when occurring in acutely critically ill patients<sup>416</sup> or in patients with chronic conditions such as hemodialysis.<sup>417</sup> This translates to an important social and economic burden: Inpatients with *S. aureus* infection had a hospital stay that was three times longer (14.3 vs. 4.5 days), charges that were three times higher (US \$48,824 vs. \$14,141), and a risk of in-hospital death that was five times greater (11.2% vs. 2.3%).<sup>418</sup> Additional costs incurred because of methicillin resistance were estimated in 2015 to be approximately \$3.3 billion yearly for US intensive care units (ICUs).<sup>419</sup>

### **Clinical Spectrum**

*S. aureus* is responsible for an array of infections wherein it is either present at the infection site or acts at a distance by secreting toxins (see “[Regulation and Virulence Determinants](#)” section). Incidence rates range between 28.4 and 35.4 per 100,000 inhabitants per year<sup>413,420</sup>; it causes mainly skin and soft tissue infection (40%), lower respiratory tract infection (20%), and BSI (20%).<sup>421</sup>

A rising incidence of *S. aureus* health care–related infections has been observed and can be attributed to medical progress.<sup>422</sup> The increasing number of invasive procedures and the use of inserted or implanted foreign bodies has indeed created a unique niche for this versatile bacterium, which is very well equipped with numerous virulence factors (see “[The Journey to Invasive Disease](#)” earlier). Accordingly, *S. aureus* is a leading cause of surgical site infections (SSIs; 20%–45% of cases),<sup>409,423</sup> catheter-related bloodstream infections (CRBSIs; 13%–40% of cases),<sup>409,424</sup> and ventilator-associated pneumonia (25%–28% of cases).<sup>409,425</sup>

In the community, it is also one of the primary causes of native and prosthetic valve endocarditis (23%–30% of cases)<sup>422,426–428</sup> and the leading organism responsible for osteoarticular infections (40%–70% of cases).<sup>429,431</sup> Finally, *S. aureus* is the second most frequent cause of community-onset bacteremia after *Escherichia coli* (15%–23.5%).<sup>432</sup>

## **Risk Factors for *Staphylococcus aureus* Infection**

Population-based studies have consistently identified male and very young and elderly individuals as being at increased risk for *S. aureus* infections. Moreover, two studies showed that the most important risk factor is necessity for dialysis, either peritoneal (relative risk [RR], 150–204) or hemodialysis (RR, 257–291). Other conditions that increase the risk of invasive *S. aureus* infections include diabetes (RR, 7), cancer (RR, 7.1–12.9), rheumatoid arthritis (RR, 2.2–9.2), human immunodeficiency virus (HIV) infection (RR, 23.7), intravenous drug use (RR, 10.1), and alcohol abuse (RR, 8.2; [Table 194.7](#)).<sup>420,433</sup>

Rare but classic predisposing factors also encompass chemotactic and phagocytosis defects. Inheritable chemotactic defects include Job syndrome, Chédiak-Higashi syndrome, Wiskott-Aldrich syndrome, and Down syndrome. *Job syndrome* is a condition that involves recurrent eczema with repeated skin infections and cold abscesses. *Chédiak-Higashi syndrome* is defined clinically by albinism and recurrent *S. aureus* infections and cytologically by giant granules in phagocytic and other cells. Acquired chemotactic defects are also relatively rare and include rheumatoid arthritis and decompensated acidotic diabetes mellitus. Opsonic defects, whether inherited or acquired, are predisposing factors for all kinds of pyogenic infections and are not specific for *S. aureus*. They are exemplified by selective or combined hypogammaglobulinemias and various kinds of complement defects.

However, one of the most important factors that independently adds to all these predisposing conditions is chronic *S. aureus* nasal carriage (see later discussion). Whether they are in the hospital or in the community, patients mostly become infected with their own carriage strain.<sup>24</sup> Therefore it has been proposed that patients at high risk for *S. aureus* nasal or cutaneous carriage should be screened, and in positive cases should be decontaminated with mupirocin ointments or other means ([Table 194.8](#)).<sup>434</sup> Implementation of this policy for selected patients admitted to the hospital should be considered.<sup>26</sup>

## **The Burden of Antibiotic Resistance**

At the beginning of last decade, MRSA was one of the most commonly identified antibiotic-resistant pathogens in many parts of the world.<sup>247,421</sup> During the period from 2000 to 2005, the proportion of hospital-onset *S. aureus* infections that were methicillin resistant peaked to 56.2% in US hospitals,<sup>435</sup> a proportion that was even higher (64.4%) in US ICUs. For some years now, surveillance programs from various areas have reported a steady decrease in the incidence of MRSA infections in the community<sup>436</sup> and in health care<sup>437</sup> or military settings,<sup>438</sup> the largest decrease being observed among hospital-onset MRSA infections<sup>411</sup> (see “[Methicillin-Resistant \*Staphylococcus aureus\*](#)” earlier).

In contrast to the diversity of strains observed in diseases caused by MSSA, MRSA outbreaks are caused by a limited number of successful clones.<sup>439,440</sup> In the health care setting, HCA-MRSA infections are associated with greater lengths of stay,<sup>441</sup> higher mortality,<sup>442</sup> and increased costs.<sup>419</sup> Whether MRSA is more virulent than MSSA is still a matter of debate. The molecular typing of thousands of carriage- and disease-associated *S. aureus* strains revealed that MRSA did not represent specific lineages and that all types of *S. aureus* can become invasive given the appropriate circumstances.<sup>443</sup> On the other hand, most MRSA infections are mainly of nosocomial origin and manifest as complications of health care procedures or underlying disorders. In this specific context, patient differences could account for the variation in mortality because a greater number of older patients with severe underlying disease contract MRSA infections.<sup>442</sup> In addition, ineffective or delay in effective antibiotic therapy could also play a large role in suboptimal response to therapy.<sup>444</sup>

CA-MRSA has now become the most frequent cause of SSTIs acquired in the community.<sup>445</sup> Groups with high-intensity physical contact are particularly affected. This includes competitive athletes, children in daycare centers, military recruits, injections drug users, jailed inmates, and men who have sex with men.<sup>254</sup> Five percent to 10% of CA-MRSA infections are invasive and can cause severe, sometimes fatal disease, such as necrotizing pneumonia, bacteremia, or necrotizing fasciitis<sup>446</sup> (see “[Methicillin-Resistant \*Staphylococcus aureus\*](#)” earlier). Successful CA-MRSA clones have established themselves as nosocomial pathogens and are now a common cause of health care-associated

**TABLE 194.7 Risk of Invasive *Staphylococcus aureus* Infection Associated With Selected Underlying Conditions in Adults 20 Years Old or Older**

UNDERLYING CONDITION	NO. OF PATIENTS WITH INVASIVE <i>S. AUREUS</i> INFECTION (N = 226)	ANNUAL INCIDENCE, PER 100,000	RR (95% CI)	P VALUE
Hemodialysis	24	7692	257.2 (161.0–393.6)	<.001
Peritoneal dialysis	3	4918	150.0 (30.5–44.1)	<.001
HIV infection	4	778	23.7 (6.4–61.4)	<.001
Solid-organ transplantation	3	683	20.7 (4.2–61.3)	<.001
Heart disease	114	362	20.6 (15.8–27.0)	<.001
Cancer	47	348	12.9 (9.1–17.8)	<.001
Illicit intravenous drug use	13	321	10.1 (5.3–17.7)	<.001
Alcohol abuse	31	241	8.2 (5.4–12.0)	<.001
Diabetes mellitus	48	192	7.0 (5.0–9.7)	<.001
Stroke	16	200	6.4 (3.6–10.6)	<.001
Chronic obstructive pulmonary disease	26	120	3.9 (2.5–5.9)	<.001
Systemic lupus erythematosus	2	80	2.4 (0.3–8.7)	.3
Rheumatoid arthritis	5	74	2.2 (0.7–5.3)	.1

CI, Confidence interval; HIV, human immunodeficiency virus; RR, relative risk.

Modified from Laupland KB, Church DL, Mucenski M, et al. Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. J Infect Dis. 2003;187:1452–1459.

**TABLE 194.8 Example of Decontaminating Scheme for Patients Colonized or Infected With Methicillin-Resistant *Staphylococcus aureus* (MRSA)**

#### Protective Measures

- Put patient in contact isolation (one or several contaminated patients in single room with restricted access)
- Use protective gown and gloves
- Use protective mask and glasses if risk for splashing of contaminated liquids
- Clean hands with alcoholic solution at glove removal and between caregiving procedures
- Leave any disposable item in room and discard for sterilization in special containers

#### Decontamination Measures

- Apply nasal mupirocin (2%) every 8 hours for 5–7 days
- Apply chlorhexidine-based oral spray three to four times a day for 5–7 days
- Take daily shower or clean body thoroughly with chlorhexidine-based soap for 5–7 days
- In the case of dental prostheses, clean and soak the prosthesis daily in chlorhexidine-based solution for 5–7 days

#### Control Cultures and Decision

- Take control swabs of any contaminated sites 48 and 96 hours after the end of treatment
- Keep isolation measures in force until laboratory results are available
- If no MRSA is present in control cultures, consider patient decontaminated; discontinue isolation, and swab weekly for follow-up cultures.
- If MRSA is present in control cultures, pursue isolation measures and repeat whole decontamination scheme

Modified from Current Recommendations at the University Hospital of Lausanne, Switzerland.

hospital-onset infections, especially BSIs.<sup>447,448</sup> Compared with HCA-MRSA, CA-MRSA retained susceptibility to many non-β-lactam antibiotics. If hospital-associated MRSA infections appear to be on the decline, only modest changes have been reported for CA-MRSA so far.<sup>449</sup>

#### Carriage of *Staphylococcus aureus*

The primary reservoirs of *S. aureus* are the anterior nares, but the organism can be isolated from other sites, especially the throat.<sup>450</sup> Traditionally, three patterns of carriage have been distinguished: persistent carriers (20% of healthy people, range 12%–30%), intermittent carriers

(30% of healthy people, 16%–70%), and noncarriers (50% of healthy people, 16%–69%).<sup>20</sup> Persistent carriers differ markedly from others: They carry the same strain for extended periods of time, have higher *S. aureus* loads, and are at higher risk of acquiring *S. aureus* infections.<sup>22,451</sup> Because intermittent carriers and noncarriers share similar characteristics, such as a low risk of infection, identical *S. aureus* nasal elimination kinetics, and comparable antistaphylococcal antibody profiles, it has been proposed that *S. aureus* carriage be reclassified such that only persistent carriers are distinguished from others.<sup>451</sup>

Bacterial and host determinants for *S. aureus* carriage have been thoroughly investigated.<sup>452</sup> In contrast to toxins, staphylococcal cell surface-associated and immune evasion molecules seem to be important for colonization efficacy<sup>453</sup> (see “The Journey to Invasive Disease” earlier). If some results suggest a role for cell wall teichoic acid, SdrC, SdrD, and SasG in binding to squamous cells in vitro, only ClfB and IsdA have been shown to be associated with *S. aureus* carriage in humans.<sup>454,455</sup> Patients exposed to skin lesions have a greater risk of *S. aureus* nasal carriage. They include patients with insulin-dependent diabetes, patients undergoing hemodialysis or peritoneal dialysis, intravenous drug users, patients with recurrent *S. aureus* skin infections or atopic dermatitis, patients with HIV, and healthy patients receiving repeated injections for allergies.<sup>452</sup>

Carriage rates vary with geographic location, age, sex, and ethnicity. Most infants become colonized shortly after birth, usually with the same strain as their mother. Carriage then decreases with age (40%–60% at 2 months, and 21%–28% at 6 months), reflecting both the development of an immune response to *S. aureus* and competition between microorganisms in the nasopharynx. This is particularly true for *S. aureus* and *S. pneumoniae* because being a *S. pneumoniae* carrier is inversely associated with *S. aureus* carriage and vice versa.<sup>456,457</sup> Children and adolescents younger than 20 years seem to have higher persistent carriage rates than adults. Since 2000, the reported prevalence rate of *S. aureus* nasal colonization has decreased to 20% to 30%.<sup>458</sup> Explanations for this decline might include improved personal hygiene, changes in socioeconomic class, and smaller families.<sup>22</sup>

Traditionally, control of *S. aureus* has been focused on preventing cross-infection between patients. However, at least three sets of observations indicate that nasal carriage of *S. aureus* is an important risk factor for sepsis: persistent carriers have higher rates of infections than others<sup>22</sup>, a large proportion of nosocomial *S. aureus* infections originate from patients' own flora<sup>24</sup>; and eradication of carriage reduces nosocomial infections, especially after orthopedic and cardiosurgery-related infections.<sup>26,459</sup>

## Carriage of Methicillin-Resistant *Staphylococcus aureus*

The prevalence of MRSA colonization increased from 0.8% in 2001 to 2002 to 3% in 2011 to 2012.<sup>460,461</sup> MRSA colonization is particularly important in the hospital environment because colonized and infected patients represent the most important reservoir of MRSA in health care facilities. Factors associated with MRSA carriage at time of hospital admission include prior health care exposure (i.e., nursing home resident or hospitalization in past 12 months), prior contact with nosocomial pathogens (i.e., history of MRSA and/or vancomycin-resistant enterococci [VRE] carriage, history of *Clostridioides difficile* [formerly *Clostridium difficile*] infection), or selected comorbid conditions such as diabetes, chronic obstructive pulmonary disease (COPD), and congestive heart failure, probably because those conditions lead to repeated hospital exposure.<sup>462</sup>

Despite the increasing rate of CA-MRSA infections, the prevalence rate of MRSA among persons without typical risk factors remains relatively low, and most MRSA colonization and infection still develop among those who have health care-associated risk factors or contact with other persons who have such risks. When patients known to be colonized with nosocomial MRSA are discharged from the hospital or nursing home into the community, spread to family members or close contacts can occur. Rates of transmission between positive case patients and household members range from less than 10% to 43%. About one-half of MRSA carriers are colonized for 2 months or less, but an estimated 20% are persistent MRSA carriers who remain positive for months or years.<sup>463</sup>

### Preventive Measures to Limit Health Care-Associated Infections Methicillin-Resistant *Staphylococcus aureus* Infections

Health care-associated infections is a leading cause of preventable illness. As a major cause of such infections, MRSA has been given priority in infection-control strategies; the United Kingdom and several US states have even mandated anti-MRSA strategies. Typical methods to limit infections by MRSA include a comprehensive set of interventions. Infection-control measures should always involve the laboratory. Determining the clonality of MRSA recovered from several patients is important in order to differentiate sporadic cases of MRSA from more problematic epidemic situations (see “**Molecular Typing**” earlier).

Improving the rational use of antibiotics and the implementation of standard precaution and hand hygiene are clearly cornerstones of MRSA prevention and control. Other anti-MRSA bundle measures (e.g., systematic active screening using either rapid or conventional testing, isolation and decolonization of carriers; see Table 194.8) when implemented routinely in settings with endemic MRSA are effective, but their use is a matter of debate because they are economically not cost-effective.<sup>464–466</sup>

Screening and isolation of MRSA carriers at admission is a resource-consuming protective measure still widely used worldwide. Both rapid PCR tests and standard culture methods can help decrease MRSA infection rates in hospitals, particularly in settings with hyperendemic MRSA cross-infections.<sup>465</sup> Decolonization of MRSA carriage has been an important part of the control of MRSA dissemination so far. Most decontamination regimens recommend a 1-week daily total body washing with a chlorhexidine-based soap, plus nasal mupirocin application (see Table 194.8). Low-level mupirocin resistance (MIC, 8–256 mg/L) and high-level resistance (MIC, >512 mg/L) exist but are uncommon and usually follow prolonged administration.<sup>467</sup> Monitoring susceptibility is essential to detect appearance of resistance to the decolonizing agent.

Recent Dutch guidelines, which use an aggressive “search and destroy” strategy, achieved a rate of up to 80% of eradication.<sup>468</sup> They differentiate between uncomplicated carriers, who have no associated MRSA infections, and complicated carriers, who may have skin or deeper infection. Uncomplicated carriers are treated only with topical measures (see Table 194.8), whereas complicated carriers receive concomitant systemic antibiotic such as TMP-SMX (160/800 mg, orally q12h) plus rifampin (600 mg orally q12h) for 1 week.<sup>468</sup> Nevertheless, eradication of MRSA carriage is often difficult, and the role of MRSA decolonization in the infection-control measure remains uncertain.<sup>459</sup>

About 5% of health care workers become colonized with MRSA, and it has long been recommended to decolonize them.<sup>469</sup> However, health care workers most frequently act as vectors of transmission, not as main sources of MRSA. A study reported in 2017 demonstrated the limited role of the environment and health care workers in transmission of *S. aureus* to patients.<sup>458</sup> The worrying trend of increasing rates of MRSA has now been reversed in many high-income countries. In this context, should we still target high-risk pathogens, or should we instead protect patient populations that are susceptible to infection from many health care-associated pathogens? Indeed, universal practices such as hand hygiene and chlorhexidine body washings<sup>470</sup> or specific measures to reduce the rate of CRBSIs including use of chlorhexidine sponges<sup>471,472</sup> helped decrease the rates of infection not only with MRSA but also with other pathogens.<sup>473</sup> Infection-control measures should therefore be regarded as a whole and should not target only a specific pathogen.<sup>474</sup>

## CLINICAL SYNDROMES

Infection begins with the colonization of target tissues by the microbes. Further spread results from more specific invasion processes, during which bacteria interact directly or indirectly (e.g., via toxins) with the host. Thus, any localized infection has the potential to become the seeding site of a more severe infection by means of contiguous extension, distant spread through the blood circulation, or production of toxins including TSST-1 and PVL (see “**The Journey to Invasive Disease**” earlier). Pyogenic infections are described subsequently

### Skin and Soft Tissues Infections

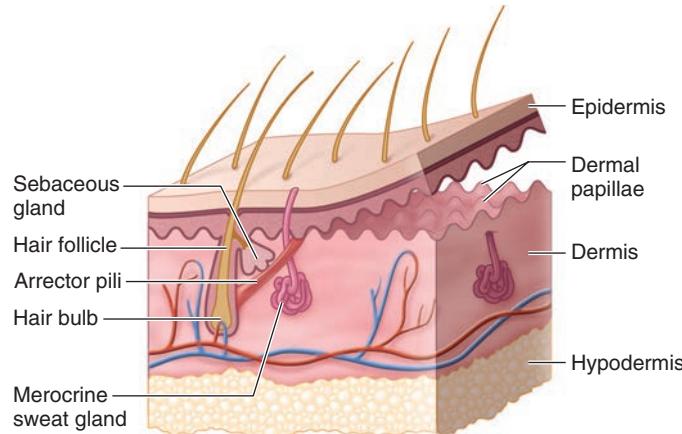
#### Classification

*S. aureus* SSTIs include primary pyoderma (such as folliculitis, furuncles, carbuncles, and impetigo) and soft tissue infections (i.e., cellulitis, erysipelas, and pyomyositis). They are commonly classified according to the anatomic structure involved (Fig. 194.14): (1) infection of the epidermis—impetigo; (2) infection of the superficial dermis—folliculitis; (3) infection of deep dermis—furuncles, carbuncles, and hidradenitis suppurativa; and (4) infection of subcutaneous cellular tissues—erysipelas, cellulitis, fasciitis, and pyomyositis.

The diagnosis of an *S. aureus* SSTI is most frequently made clinically. The basic anatomic lesion induced is a pyogenic exudate or an abscess. Superficial infections can often be treated with local care, surgical drainage, and rarely, systemic antibiotics. When the infection penetrates to the deeper subcutaneous tissue and/or surgery is required, it is considered complicated; erysipelas, lymphangitis, lymphadenitis, cellulitis, and necrotizing fasciitis are severe diseases that may be life-threatening. They require hospitalization, systemic antibiotic therapy, and prompt surgical drainage and débridement<sup>184</sup> (Tables 194.9 and 194.10).

#### Impetigo

Impetigo is a superficial infection of the skin that involves only the epidermis. It affects mostly children, usually on exposed areas of the



**FIG. 194.14** Skin anatomy delineating various levels at which *Staphylococcus aureus* infection can occur (see text for details).

**TABLE 194.9 Initial Management of Adults With Community-Acquired Purulent Skin and Soft Tissue Infections Presumed to Be Staphylococcal (Dosages Given for Patients With Normal Renal Function)**

1. Incise and drain purulent foci. Consider heat, elevation, and immobilization of site.
2. Obtain wound Gram stain, culture, and two blood cultures with susceptibility testing.
3. Consider admitting for observation patients who require extensive débridement, have large areas of erythema, are elderly, are immunosuppressed, have important comorbidities (such as chronic renal failure, morbid obesity, diabetes mellitus), are unable to take medication reliably, or who are clinically unstable.
4. Administer intravenous therapy for hospitalized patients
  - A. Initially cover MRSA with
    - a. Vancomycin: 15 mg/kg IV q12h
    - b. Alternatives include:
      - i. Daptomycin: 6 mg/kg IV q24h
      - ii. Ceftaroline: 600 mg IV q12h
  - B. If cultures grow MSSA, can switch to:
    - a. Oxacillin, nafcillin, or flucloxacillin (not in United States): 2 g IV q4h, or
    - b. Cefazolin: 2 g IV q8h
5. Patients appropriate for outpatient therapy (with follow-up scheduled for 48–72 h): trimethoprim-sulfamethoxazole—2 double-strength tablets (320/1600 mg orally q12h)
 

Alternatives include:

  - A. Clindamycin: 300–450 mg orally q8h
  - B. Doxycycline or minocycline: 100 mg orally q12h
  - C. Linezolid: 600 mg orally q12h
  - D. Tedizolid: 200 mg orally q24h
  - E. Oritavancin: 1.2 g IV over 3 h once, or dalbavancin 1 g IV over 30 min, followed by 500 mg IV 1 week later
  - F. If cultures grow MSSA can switch to:
    - a. Dicloxacillin: 500 mg orally q6h
    - b. Cephalexin: 500 mg orally q6h

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



**FIG. 194.15** Infective skin lesions in staphylococcal impetigo.

body (e.g., the face and the legs). Primary impetigo occurs via direct bacterial invasion of intact healthy skin, whereas secondary impetigo occurs via infection at sites of minor skin trauma such as abrasions, insect bites, or eczema. Most cases of impetigo are caused by *S. aureus*, *S. pyogenes* or mixed infections of staphylococci and streptococci.<sup>475</sup>

The disease usually starts as a red macule that evolves into vesicles that contain cloudy fluid based on the area of erythema (Fig. 194.15). Bullous impetigo is mostly exclusively due to *S. aureus* strains producing exfoliative toxins (see “**Exfoliative Toxins and Staphylococcal Scalded Skin Syndrome**” earlier). The bullae are the result of epidermolytic toxins of the serine protease family. The vesicles rapidly rupture and leave a yellowish, thick, wet crust with a diameter exceeding 1 cm that is surrounded by erythema. Most affected children present with multiple

lesions of various ages. General symptoms are absent, but a local inflammatory lymph node reaction is a rule. At the beginning, the differential diagnosis includes other vesicular eruptions, such as herpes simplex and varicella. However, the evolution is typical and rapidly differentiates the diseases. Although of mild severity, the disease is extremely contagious and the affected child should be kept apart from other children until an effective treatment has been applied. A basic treatment that combines disinfection with a chlorhexidine-based or povidone-iodine-based soap and additional bacitracin zinc ointment in patients with limited lesions, or fusidic acid cream (not available in the United States) in cases of more extensive lesions, is generally sufficient. Oral antibiotics are rarely needed. Mupirocin should be reserved for the treatment of *S. aureus* nasal carriage.

### Folliculitis

Folliculitis is defined as a pyoderma that involves the hair follicle and its immediate surroundings. It manifests as a series of raised painful reddish lesions with indurated bases, each of them centered on a hair follicle. Extensive folliculitis of the bearded area of the face is called *sycosis barbae*. General symptoms are usually absent, and local antiseptic measures are the treatment of choice.

### Furuncles and Carbuncles

Furuncles (boils) represent extension of the infectious process involving the hair follicle and are located, by definition, on the hairy areas of the body, with a predilection for the face, neck, axilla, and buttocks. The disease starts as a painful red nodule and rapidly evolves into a hot, painful, raised, and indurated lesion with a diameter of 1 to 2 cm. Its evolution is characterized by the appearance of a yellowish area in its center. On rupture (either spontaneous or surgical), it liberates a small amount of yellowish creamy discharge of purulent and necrotic material. Secondary foci from autoinoculation are frequent. General symptoms are normally absent. Local treatment is usually sufficient. In case of recurrent episodes, testing for nasal carriage and appropriate eradication may be necessary, although its effectiveness in reducing the risk of recurrence is not clearly shown.

CA-MRSA is a particular issue in furuncles.<sup>254,445</sup> They are often centered by a necrotic spot, are multiple, and occur in outbreaks. Lesions can progress to abscesses and cellulitis. In young patients with boils and systemic signs of infection, one must remember the risk of severe hemorrhagic pneumonia (see Fig. 194.8) or necrotizing fasciitis<sup>446</sup> (discussed in “**The Journey to Invasive Disease**” earlier).

Another remarkable situation is when furuncles are located around the nares or upper lip. Such lesions may lead to life-threatening septic thrombophlebitis of the cavernous sinus. Therefore, furuncles in this location should be treated with high-dose parenteral antibiotics.

Carbuncles are deep-seated infections that involve several hair follicles and result from the coalescence and spreading of the infectious process into the depths of subcutaneous tissue. They are usually localized at the base of the neck. The disease leads to the development of a central necrotic crater, which heals with the development of a hard hypertrophic violaceous scar. Fever and malaise are generally present. Carbuncles may be the source of bacteremia and require parenteral antibiotic therapy.

### Hidradenitis Suppurativa

Hidradenitis suppurativa is a pyogenic infection of the apocrine sweat glands that manifests as crops of furuncles that develop in the axillary, perineal, and genital areas. After spontaneous drainage, hypertrophic scarring may occur. As in furunculosis, treatment is primarily limited to local care and topical disinfectants. Administration of oral antimicrobials is indicated only in the case of systemic symptoms.

### Mastitis

Symptoms of *mastitis* occur in up to 25% of mothers in the United States who are breastfeeding, but the incidence of staphylococcal mastitis necessitating therapy is approximately 2.5%.<sup>476</sup> The infection develops most commonly during the second or third week of the puerperium. The diagnosis of mastitis is usually clinical, with patients presenting with focal tenderness in one breast accompanied by fever and malaise.

**TABLE 194.10 Suggested Therapy for Native Valve and Prosthetic Valve Endocarditis Caused by *Staphylococci***

ANTIBIOTIC	FREQUENCY, DOSAGE, AND ROUTE	DURATION	COMMENTS
<b>Native Valves</b>			
<b><i>Methicillin-Susceptible Staphylococci</i></b>			
Flucloxacillin (not in United States) or oxacillin or nafcillin	2 g IV q4h	4–6 wk	Addition of gentamicin no longer recommended for native valve endocarditis
Cefazolin (or other first-generation cephalosporins) <sup>a</sup>	2 g IV q8h	4–6 wk	Alternative for patients allergic to penicillins (not in case of immediate-type penicillin hypersensitivity)
<b><i>Methicillin-Resistant Staphylococci</i></b>			
Vancomycin	15–20 mg/kg IV q12h	4–6 wk	
Daptomycin	8–10 mg/kg IV q24h	4–6 wk	
<b>Prosthetic Valves</b>			
<b><i>Methicillin-Susceptible Staphylococci</i><sup>b</sup></b>			
Flucloxacillin (not in United States) or oxacillin or nafcillin	2 g IV q4h	≥6 wk	
with rifampin	900 mg IV q24h or 300 mg orally q8h	≥6 wk	Rifampin increases hepatic metabolism of numerous drugs, including warfarin
and gentamicin	3 mg/kg IV q24h or 1 mg/kg IM q8h	2 wk	
<b><i>Methicillin-Resistant Staphylococci</i></b>			
Vancomycin	15–20 mg/kg IV q12h	≥6 wk	
plus rifampin	900 mg IV q24h or 300 mg orally q8h	≥6 wk	
and gentamicin	3 mg/kg IV q24h or 1 mg/kg IM q8h	2 wk	

<sup>a</sup>American Heart Association.

<sup>b</sup>Rifampin plays a special role in prosthetic device infection because it helps kill bacteria attached to foreign material. Rifampin should never be used alone because it selects for resistance at a high frequency (about 10<sup>-6</sup>).

Modified from Que YA, Moreillon P. Infective endocarditis. Nat Rev Cardiol. 2011;6:322–336; and Baddour LM, Wilson WR, Bayer AS, et al. 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. 2015;132:1435–1486.

Treatment includes analgesics, changing breastfeeding technique, and reversing milk stasis, often with the assistance of a lactation consultant. Continued breastfeeding should be encouraged in the presence of mastitis and generally does not pose a risk to the infant. Evidence has suggested that mastitis can be either prevented<sup>477</sup> or treated<sup>478</sup> with probiotics such as lactobacilli. When antibiotics are needed (i.e., in the presence of acute pain, systemic symptoms, or fever), those effective against *S. aureus* are preferred. Breast abscess is the most common complication of mastitis. It can be prevented with early treatment of mastitis and continued breastfeeding. Once an abscess occurs, surgical drainage or needle aspiration is needed.

#### Surgical Site Infection

SSIs are infections that occur at or near the incision site within 30 days of the procedure, or within 90 days if prosthetic material is implanted at surgery; they account for 20% to 30% of all hospital-acquired infections.<sup>410</sup> The commonly reported incidence rate of 2% to 5% of patients undergoing inpatient surgery is probably underestimated because of inadequate postdischarge surveillance.<sup>479</sup> *S. aureus* is the most prevalent pathogen that causes SSI for most types of surgery (15%–45% of cases),<sup>409,410,423</sup> especially after clean procedures. Gram-negative rods and enterococci are, however, the most prevalent pathogens that cause SSI after abdominal surgery.<sup>435</sup> Most infections are caused by *S. aureus* strains that are carried by the patient at admission to the hospital.<sup>24</sup> Hence, nasal carriage of *S. aureus* is a risk factor for subsequent infection in patients undergoing surgery, and decolonization has been recommended, especially for patients undergoing orthopedic or cardiothoracic procedures.<sup>480</sup>

SSIs are characterized by progressive edema, erythema, and pain around the surgical incision. General symptoms are frequently associated. Careful inspection of the wound, ulcer, or lesion is essential. If deeper

structures are not involved, release of the stitches, repeated cleansing, and antibiotic coverage for 7 to 10 days is usually curative. If the infection involves deeper structures (e.g., bone) or foreign material (e.g., prosthetic devices), prolonged parenteral antibiotic therapy (4–6 weeks) may be necessary, and removal of the foreign material is warranted.<sup>184</sup> The evolution of wound infection is highly dependent on the patient's comorbidities. Healing may be delayed, particularly in vascular insufficiency and diabetes.

#### Erysipelas, Cellulitis, and Fasciitis

Superficial or deep extension of infection may result in erysipelas, cellulitis, and fasciitis. Erysipelas and fasciitis are commonly the result of *S. pyogenes*, but not exclusively. Fasciitis can arise from hematogenous seeding. An important common feature of all three entities is severe pain. In the case of erysipelas, soreness is associated with typical skin lesions. In the case of cellulitis or fasciitis, on the other hand, severe pain symptoms are disproportional with regard to the visible anatomic lesions (Fig. 194.16). Hence, high fever, severe local pain, and relatively meager clinical findings at visible examination are highly suggestive of one of these entities. The consideration is important because emergency surgical drainage is indicated in the case of fasciitis, and prompt intervention may be delayed because of the confounding picture.

Erysipelas is a superficial cellulitis with prominent lymphatic involvement, with an indurate, “peau d'orange” appearance with a raised border that is demarcated from normal skin. It often complicates edematous extremities and skin ulcers such as in varicose limbs. As in impetigo, it may be the result of mixed *S. pyogenes* and *S. aureus* infection. Clinical signs of sepsis with high fever are present. In most cases, an etiologic diagnosis is not established. Local sampling with puncture may be attempted, and blood cultures must be drawn. Prompt empirical treatment



**FIG. 194.16 *Staphylococcus aureus* cellulitis of the elbow in cancer patient with low neutrophil counts.** Pain was disproportional to visual appearance of the lesion. Patient was bacteremic.

should be started with parenteral antibiotics covering at least both staphylococci and streptococci.

In patients with underlying conditions, such as diabetic foot, mixed pictures of erysipelas and cellulitis may occur and may also be the result of gram-negative bacteria, including *P. aeruginosa*. Therefore the spectrum of empirical treatment should be broadened to cover these agents until the microbiologic results are available. If gram-negative bacteria or MRSA is suspected, the addition of aminoglycoside or vancomycin to a broad-spectrum β-lactam may be warranted.

Cellulitis involves deeper anatomic structures and does not produce the typical geographic skin lesion of erysipelas (see Fig. 194.16). Therefore it is more confusing and may be mistaken for nonspecific lesions such as trauma. Associated pain and fever are important signs. Cellulitis may be from multiple other organisms, including gram-negative bacteria, especially in patients with immunocompromise. Therefore, microbiologic sampling (including blood cultures) should be promptly followed by broad-spectrum antibiotic therapy with both anti-gram-positive and anti-gram-negative coverage. Radiologic examination is unnecessary in most cases of cellulitis. Computed tomography (CT) is useful when subjacent osteomyelitis is suspected, whereas magnetic resonance imaging (MRI) helps to differentiate cellulitis from necrotizing fasciitis and osteomyelitis from adjacent soft tissue infection.

Necrotizing fasciitis is the most severe condition and paradoxically causes the least superficial signs at visual observation of the skin and soft tissues. The pain may be so intense that opiate administration is required for relief. The condition is often the result of *S. pyogenes*, but *S. aureus* may be involved, especially in the presence of CA-MRSA.<sup>254</sup> Gram-negative bacteria, including *P. aeruginosa*, may be responsible, especially in patients with immunocompromise, and must be considered in the choice of initial empirical treatment.

Whatever its cause, fasciitis is an absolute emergency that necessitates immediate and generous surgical débridement and drainage. The evolution is a matter of minutes rather than hours and may rapidly result in amputation or death. Prompt clinical diagnosis and multidisciplinary evaluation are warranted. Imaging may help delineate the lesions, but urgent surgical exploration and fasciotomy should not be delayed. High-dose and broad-spectrum antibiotic therapy is necessary and can be readjusted after the bacterial pathogen has been isolated. In the case of *S. pyogenes* and severe refractory shock, compassionate use of intravenous immunoglobulin has been proposed<sup>186</sup> (see also “Non-menstrual Toxic Shock Syndrome” earlier).

### Management of Skin and Soft Tissue Infection

Localized lesions can be handled with disinfectant or topical antibiotics such as fusidic acid. However, increasing rates of resistance to topical agents have been reported. Mupirocin should be reserved for the

treatment of *S. aureus* nasal carriage. Moderate and severe SSI should be treated with systemic antibiotic. A decision to hospitalize a patient is made based on clinical judgment (large abscesses, signs of systemic infection) and the at-risk characteristics of the patients (age <6 months, diabetes, or immunodeficiency). Surgical drainage is a major part of treatment, and most benign SSTIs are cured with drainage alone.<sup>184</sup>

Empirical therapy of health care-associated SSTI should cover multiresistant HCA-MRSA and thus include vancomycin or maybe linezolid. Empirical therapy of community-acquired SSTI is complicated by the occurrence of CA-MRSA. β-Lactams should be used with caution and according to the local epidemiology. Unlike HCA-MRSA, CA-MRSA is often susceptible to clindamycin and TMP-SMX, sometimes to tetracyclines, and mostly to linezolid. However, if coinfection with *S. pyogenes* is found and antibiotics are required, linezolid, vancomycin, or maybe tigecycline should be considered for first-line treatment. If toxin secretion is an issue, linezolid might be preferred.<sup>481</sup>

### Bloodstream Infection

BSI is defined as one or several positive blood cultures associated with general symptoms such as fever or hypotension.<sup>482</sup> Its incidence rate increased from 7.4 episodes per 1000 admissions in the 1950s<sup>483</sup> to 31.2 episodes per 1000 admissions in 2006.<sup>484</sup> Population-based studies have estimated the rate of BSI to be around 140 to 160 BSIs per 100,000 inhabitants/year in high-income countries.<sup>482</sup> The most common isolates are *E. coli* and *S. aureus*, with incidence rates estimated at 30 to 50 per 100,000 inhabitants/year and 20 to 35 per 100,000 inhabitants/year, respectively.<sup>482,485</sup> Higher incidence rates of *S. aureus* BSI are observed in countries with a greater burden of MRSA, in men, or at either extreme of life (<1 year and >75 years). Mortality of *S. aureus* BSI (15%–25%) has stabilized since the 1990s and varies as a function of age and underlying conditions.<sup>414</sup> Despite the increased prevalence of SSTI from CA-MRSA in outpatients in the United States, a parallel increase in CA-MRSA bacteremia or endocarditis has not been reported.<sup>485</sup> Studies have suggested that PVL-positive strains are more likely to infect skin and soft tissues (see previous section).

BSI is usually divided into two categories: nosocomial BSI, wherein positive blood cultures occur 2 days or more after hospital entry; and community-acquired BSI, which occurs in the community or before 2 days of hospitalization. However, the increasing number of individuals treated in outpatient programs makes these two categories progressively overlapping. Hence, community-acquired BSI may be more appropriately referred to as *community-onset BSI*, which is further subdivided into *health care-associated BSI* (HCA-BSI) and *community-associated BSI* (CA-BSI). The subdivision is similar to that made between HCA-MRSA and CA-MRSA.

### Community-Onset Bacteremia

Community-onset HCA-BSI is comparable with nosocomial BSI in terms of risk factors of multiresistant organisms. These include intravenous devices, a history of surgical treatment, and dialysis. In contrast, community-onset CA-BSI, which occurs in patients without underlying conditions, is mostly from antibiotic-susceptible organisms and always associated with a detectable infected focus, including SSTI, deep-seated abscesses, pneumonia, osteoarticular infections, or infective endocarditis.<sup>486</sup> Of note, patients on dialysis are at particularly high risk for staphylococcal endocarditis and represent a distinct at-risk group for this disease.<sup>426,487</sup>

### Nosocomial and Health Care-Associated Bloodstream Infection

*S. aureus* is the leading cause of nosocomial BSI and HCA-BSI, together with CoNS.<sup>409,410</sup> These are mostly associated with the presence of intravascular catheters or devices, procedures in contaminated sites, SSI, and sometimes *S. aureus* pneumonia.<sup>409</sup> Complications involve peripheral metastatic foci, which can reveal themselves later. Nosocomial *S. aureus* bacteremia enters in the differential diagnosis of any hospital-related febrile or septic episodes. The risk for patients with catheter-induced *S. aureus* bacteremia to develop infective endocarditis is about 10%.<sup>280,488</sup> Thus, catheter-related *S. aureus* bacteremia must be taken very seriously and consideration given to excluding infective endocarditis with transesophageal echocardiography.<sup>489–491</sup>

## Management of *Staphylococcus aureus* Bloodstream Infection

With *S. aureus*, even a single positive blood culture should prompt initiation of antibiotic therapy, sampling of blood for follow-up cultures, determination of the source and extent of infection, and finally search for endocarditis with transesophageal echocardiography.<sup>490</sup> Approximately one-third of patients with *S. aureus* BSI develop metastatic complications, especially in cases involving prosthetic material. The strongest indicators of clinical complication are a positive result of follow-up blood culture after 48 to 96 hours of treatment and persistent fever at 72 hours.<sup>492</sup>

Removal of the original focus is a golden rule, especially in the case of infected intravascular material or prosthetic devices. Failure to do so is strongly associated with recurrence. In the case of difficult-to-remove implanted catheters, the use of antibiotic locks between infusions may be attempted, but success is variable.<sup>493</sup> In case of a removable infection source (e.g., a catheter), a 10-day to 14-day post-catheter removal antibiotic treatment may be appropriate: (1) after the removal of all prosthetic material and endovascular catheter; (2) after the exclusion of endocarditis; (3) as long as the follow-up blood cultures drawn 2 to 4 days after initial positive cultures are negative for *S. aureus*; (4) if the fever has vanished within 72 hours after the initiation of antistaphylococcal therapy; and (5) when the absence of metastatic foci has been confirmed.<sup>493,494</sup>

Although no consensus recommendations exist regarding therapy, most authorities advocate a 14-day course of antibiotic treatment in case of BSI related to a removable catheter or drainable localized infection.<sup>495</sup> Deeper infections, such as arthritis, osteomyelitis, and endocarditis, must be treated with antibiotics for 4 to 6 weeks, with or without surgery depending on individual circumstances (see Chapters 80, 81, 103, 104, and 105 for more detailed recommendations). Empirical antibiotic treatment must take into account the probability of methicillin-resistant staphylococci, which may represent more than 50% of staphylococcal infections in the hospital milieu.<sup>490</sup>

## Infective Endocarditis

Infective endocarditis is one of the most severe complications of *S. aureus* bacteremia. The disease is uniformly lethal if not treated with antibiotics with or without surgery. *S. aureus* endocarditis typically follows an acute course with multiple peripheral septic emboli, valve destruction, myocarditis, and mixed cardiogenic and septic shock. Appropriate care involves a multidisciplinary evaluation, including infectious disease and microbiology experts, cardiologists, intensive care specialists, cardiac surgeons, and sometimes neurologists.<sup>489,491</sup>

## Epidemiology

In spite of improved health care, the overall incidence rate of infective endocarditis has remained at 2 to 8 cases per 100,000 population per year over the last 40 years.<sup>426–428,496</sup> Risk factors have, however, been changing. Chronic rheumatic heart disease, which was a prime risk factor in the preantibiotic era, is being replaced by other at-risk groups, including patients with age-related degenerative valve lesions, prosthetic valves,<sup>497</sup> intracardiac devices, or intravascular prostheses. Coexisting conditions such as hemodialysis,<sup>498</sup> diabetes, intravenous drug use, or HIV infection increase the risk for infective endocarditis.<sup>487</sup>

As a consequence, the epidemiology of infective endocarditis has changed; the mean age of patients with infective endocarditis has increased from 30 years in the 1950s to older than 60 years since the 1990s. The incidence of infective endocarditis is now highest in men aged 75 to 79 years.<sup>427</sup> Oral streptococci, which are still a leading cause in developing countries, have been supplanted by *S. aureus* and CoNS, especially in industrialized countries.<sup>422,427,499,500</sup> This finding also correlates with the fact that the portal of entry has become more often cutaneous or procedure related than dental. Accordingly, patients with health care-associated infective endocarditis may represent up to one-fourth to one-third of all cases of infective endocarditis in recent studies.<sup>427,499</sup>

## Pathogenesis

### Role of Bacterial Adhesins

The pathogenesis of *S. aureus* endocarditis implicates a close relationship between certain *S. aureus* surface adhesins (MSCRAMMs) and host proteins present on the surface of damaged or inflamed valves. Physically

damaged endothelia are covered by a meshwork of fibrin, platelets, and numerous host matrix proteins.<sup>501</sup>

*S. aureus* is extremely well equipped with both surface-bound and secreted factors that mediate tissue colonization and invasion (see Table 194.3 and “The Journey to Invasive Disease” earlier). With a system developed in *Lactococcus lactis*,<sup>502</sup> Que and colleagues<sup>81,503</sup> showed that *S. aureus* ClfA was necessary and sufficient for early valve colonization and infection in rats with experimental infective endocarditis but not sufficient for invasive and persistent disease. The same authors showed that fibronectin-binding protein A (FnBPA) promoted both early valve colonization and persistent infection. FnBPA is a peculiar MSCRAMM that carries at least three binding specificities, to fibronectin, to fibrinogen, and to elastin.<sup>504</sup> Construction of truncated and chimera proteins indicated that although fibrinogen binding was necessary and sufficient for early valve colonization, as observed with ClfA, fibronectin binding was necessary for further invasion and persistence. This invasive phenotype was associated with the capacity of fibronectin binding to trigger active internalization of staphylococci into eukaryotic cells, both *in vitro* and *in vivo*. Thus, valve infection proceeds through consecutive binding to fibrinogen, for early colonization, and fibronectin, for invasion and persistence.<sup>81</sup> Such interadhesin cooperation could also occur between other MSCRAMMs, adding even more flexibility to the already wealthy set of *S. aureus* surface determinants.<sup>81,82</sup>

In the case of physically intact but inflamed endothelia, *S. aureus* fibronectin-binding proteins might be of primary importance. During inflammation, endothelial cells express integrins of the  $\beta_1$  family, which can bind plasma fibronectin at the luminal pole of the cell. The resulting fibronectin coat functions as a ligand surface for circulating *S. aureus* that expresses fibronectin-binding proteins. The contact between the adhesin and its ligand triggers the active internalization of *S. aureus* by endothelial cells and by other cells.<sup>505</sup> Once internalized, *S. aureus* may either persist locally, protected from host defenses and antimicrobial therapy, or multiply and secrete hemolysins (see Tables 194.1 and 194.3), which lyse the host cell and allow bacteria to spread both locally and to distant organs. This second scenario probably explains many cases of infective endocarditis on anatomically normal valves.

### Role of Platelets

The role of coagulation is critical in funneling circulating *S. aureus* clumps toward damaged endothelia (see “Contribution of Coagulation” earlier). However, bacteria-induced platelet activation is a double-edged sword. On the one hand, platelets contribute to the formation of the vegetation. On the other hand, platelets also contribute to antiinfective host defenses by releasing AMPs and inflammatory mediators. These cationic AMPs are contained in the  $\alpha$ -granules of thrombocytes and are released into the surroundings by activated platelets. They kill numerous gram-positive organisms by perturbing their membrane potential.<sup>506,507</sup>

Experimental evidence indicates that platelet-resistant mutants of staphylococci or streptococci have an increased ability to produce endocarditis in animals. In addition, clinical studies have indicated that isolates of *S. aureus* recovered from patients with endocarditis are more often resistant to platelet-induced killing than *S. aureus* isolated from other infected sites.<sup>508</sup> Resistance of *S. aureus* to AMPs is mediated by the *dlt* and *mrp* operons (see “Immune Evasion” section earlier). Additional resistance strategies include binding and inactivation of AMPs by staphylococcal proteases such as Sak and Aur (see Table 194.1).

Experiments have confirmed that fibrinogen adherence and platelet aggregation cooperated to induce experimental endocarditis in rats.<sup>510</sup> In addition, interaction with vWF is also involved.<sup>120</sup> Finally, retrospective studies suggested that patients on prior aspirin therapy were significantly less likely to have vegetations on cardiovascular implantable electronic device leads or heart valves than those who had not received it,<sup>511</sup> yet without any impact on survival<sup>511</sup> or embolism.<sup>512,513</sup>

This raises the question regarding whether antiaggregant therapy might be useful in the prevention or treatment of infective endocarditis. Experimental antiaggregant prophylaxis is therefore being revisited in experimental models and has generated promising results.<sup>121</sup> Likewise, antiaggregant therapy with acetylsalicylic acid decreases the severity of experimental endocarditis from *S. aureus*.<sup>514</sup>

One randomized, double-blind, placebo-controlled trial showed that a daily dose of aspirin (325 mg orally q24h) in addition to the conventional antibiotic therapy did not reduce the risk of embolic events in patients with infective endocarditis and was even associated with an increased risk of bleeding.<sup>515</sup> Similarly, anticoagulants increased the risk of secondary bleeding at the site of septic emboli, including hemorrhagic stroke, and are not recommended.<sup>516,517</sup> However, these studies did not look at endocarditis prevention sensu stricto, because the studied patients had already developed valve infection and thus were more prone to have secondary bleeding. A more realistic protocol should include patients who are at risk of endocarditis and are taking or not taking prophylactic antiaggregants, and test whether or not they have a modified risk of contracting endocarditis.

### Host Defenses and Prevention

The role of host defenses is marginal in infective endocarditis. Once staphylococci have colonized the valves, their intrinsic procoagulant activities (e.g., fibrinogen polymerization by coagulase and platelet activation by fibrinogen-binding protein) trigger further deposition of platelets and fibrin on top of the microorganisms, thus providing a protective niche inside the vegetation. Moreover, *S. aureus* can be internalized into endothelial cells via bridging with fibronectin (see previous discussion). Both cases result in a failure of professional phagocytes to eradicate the organisms (see also “Immune Evasion” earlier).

Killing by T-cell-mediated effectors is not operative in endocarditis. Therefore, the only alternative is antibody-mediated protection, which could act before colonization by blocking *S. aureus* surface adhesins or by increasing the speed of blood clearance via opsonization. Active research is dedicated to such an approach, but few promising results are available yet. The limitation of preventive vaccines in endocarditis might be the short (1-minute to 2-minute) delay between blood invasion and valve colonization, which leaves a small window for antibodies to be active.

Other preventive measures are scarce. Because *S. aureus* is a ubiquitous skin colonizer, prevention starts with hygiene and disinfection. Decontamination of carriers and proper antisepsis at the site of injection and catheter placement is mandatory. Antibiotic prophylaxis may be useful but is limited to cases of medicosurgical procedures in the area of well-defined infective foci (see Chapter 83 for details). Thus, detection of patients with risk factors, and respect for proper hygiene and antisepsis measures, remain the cornerstones of *S. aureus* endocarditis prevention.

### Clinical Spectrum

*S. aureus* infective endocarditis often manifests as an acute septic syndrome with fever, tachycardia, and often hypotension. Dyspnea may be present from congestive heart and from septic pulmonary emboli in the case of right-sided endocarditis. General signs such as arthralgia or myalgia, back pain, and pleuritic pain are present in 10% to 50% of cases. Specific signs include a new cardiac murmur, usually of valve regurgitation, in about 90% of cases; septic emboli in the form of petechiae and Janeway lesions; and central nervous system manifestations in up to one-third of patients (Figs. 194.17, 194.18, and 194.19).

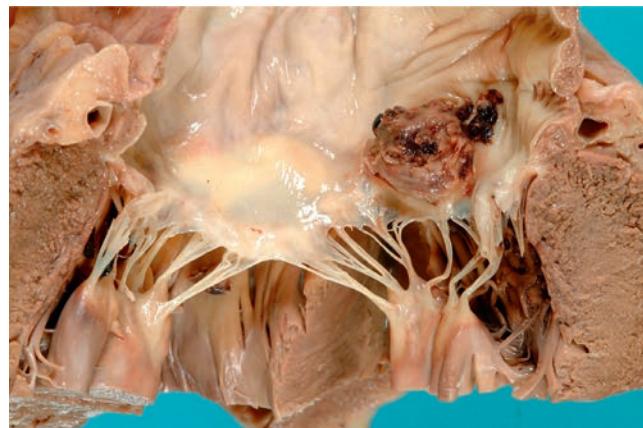
Cardiac failure is a major indication for emergency valve replacement. A defect in atrioventricular conduction may represent a mycotic aneurysm of the sinus of Valsalva, usually the noncoronary cusp. Transesophageal echocardiography is useful in detecting this complication. Large vegetations ( $\geq 1$  cm) are relatively frequent in *S. aureus* endocarditis and have been associated with an increased risk of embolization. However, the risk of embolization rapidly decreases within the first days of efficacious therapy.<sup>518</sup>

### Vascular Complications

Emolic skin lesions encompass Janeway spots, occurring in acute endocarditis and often containing bacteria (see Fig. 194.17), or more delayed immune-related vasculitis (Roth spots) that are a sign of relatively chronic infection and are not a usual feature of acute *S. aureus* endocarditis. Larger septic emboli from broken-off vegetations can occlude the coronary or peripheral arteries, especially in the brain. Mycotic aneurysms are found in up to 15% of patients with bacterial endocarditis, and probably more frequently in *S. aureus* endocarditis. They may arise either from direct invasion of the arterial wall by the infecting organisms,



**FIG. 194.17** Embolic skin lesions (Janeway spots) in framework of acute mitral valve endocarditis caused by *Staphylococcus aureus*.

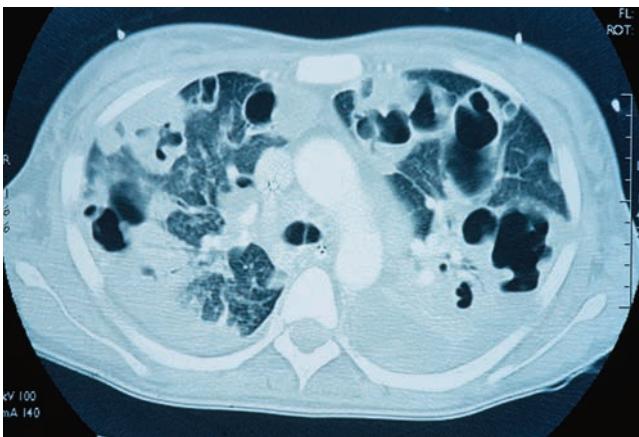


**FIG. 194.18** *Staphylococcus aureus* endocarditis of the mitral valve. Note large ulcerative lesion on anterior leaflet. (Courtesy Drs. A. Lobrinus and I. Letovanec, Pathology Institute, Lausanne University.)

from septic embolization of the vasa vasorum, or from the deposition of immune complexes that trigger local inflammation and weakening of the arterial wall.

### Neurologic Complications

Cerebral complications are frequent, occurring in 15% to 25% of patients<sup>517,519</sup> and are associated with poor outcome.<sup>520</sup> Systematic brain imaging with MRI may reveal cerebral events in up to 80% of patients; among them, 50% are asymptomatic.<sup>521</sup> Lesions include ischemic and hemorrhagic stroke, transient ischemic attacks, silent cerebral embolism,



**FIG. 194.19** Chest computed tomography scan of 30-year-old intravenous drug user with tricuspid valve *Staphylococcus aureus* endocarditis and bilateral lung abscesses and empyema.

abscesses, mycotic aneurysms, encephalopathy, and meningitis.<sup>517,519,521</sup> Control of the infection is essential because embolization sharply decreases thereafter.<sup>518</sup> Patients with large mobile mitral vegetations are at higher risk of embolism.<sup>517</sup> Recurrent embolization after the onset of efficacious therapy may be an indication for urgent valve replacement. This decision is difficult because anticoagulation therapy during extracorporeal circulation and after valve replacement puts the patients at increased risk of secondary intracerebral hemorrhage.<sup>489,517</sup> However, recent studies have indicated that early surgery (i.e., within the hospital stay) is usually beneficial.<sup>522</sup>

### Diagnosis

Criteria for the diagnosis of infective endocarditis changed with the use of echocardiography and are discussed in detail in Chapter 80. In *S. aureus* endocarditis, the first two blood cultures are positive in more than 90% of cases. The volume of blood cultures is critical because persistent bacteremia in infective endocarditis is often of low level, representing 1 to 100 bacteria per milliliter of blood. For each culture, 8 to 12 mL of blood should be drawn, with careful antiseptic precautions.

### Management of *Staphylococcus aureus* Endocarditis

Treatment options are listed in Table 194.10<sup>489,491</sup> (see Chapters 80 and 81).

### Management of Right-Sided Endocarditis in Injection Drug Users

*S. aureus* is the most common, yet not unique, pathogen in right-sided endocarditis in intravenous drug users.<sup>523</sup> Although standard treatment of left-sided *S. aureus* endocarditis requires 4 to 6 weeks of antibiotic therapy (see Table 194.10 and Chapter 80), the duration of treatment for right-sided endocarditis can be considerably shortened in injection drug users<sup>489,491,524,525</sup> because right-sided endocarditis has somewhat different physiopathologic features and is easier to cure or can heal spontaneously in experimental models. Moreover, the prognosis is less severe than in left-sided valve infection. Agents proven appropriate for 2-week treatment of right-sided endocarditis are nafcillin and oxacillin. Combination with an aminoglycoside was shown to be not necessary.<sup>524,525</sup> However, short-course therapy is limited to favorable conditions, including infection with an MSSA, prompt response to treatment, infection limited to the tricuspid valve, <20-mm vegetations, absence of extracardiac infected sites such as empyema, and absence of severe immunodepression (>200 CD4 cells/mL) in HIV-infected patients.<sup>489,491,524</sup>

Another proposed regimen that may allow intravenous-oral switch therapy is ciprofloxacin plus rifampin.<sup>526,527</sup> Today newer anti-gram-positive quinolones (e.g., levofloxacin or moxifloxacin) should be preferred to ciprofloxacin, because they are less prone to select for resistance. Some authors do not start rifampin until 3 days of effective therapy have been given, hoping to reduce the chance of secondary rifampin resistance.

Daptomycin is an alternative for *S. aureus* right-sided infective endocarditis therapy (see Chapter 31).<sup>236</sup> However high doses (10 mg/kg/24 h) should be used, and combination with oxacillin or fosfomycin to avoid the development of resistance has been suggested (see “Daptomycin” section earlier). On the other hand, glycopeptides (vancomycin or teicoplanin) should not be used for short-course treatment because of their lack of bactericidal activity and poor vegetation penetration.

## Infections of the Central Nervous System

### Meningitis

*S. aureus* meningitis is an uncommon disease that accounts for about 5% of cases of bacterial meningitis.<sup>528</sup> Two different modes of pathogenesis have been described: (1) postoperative meningitis, associated with neurosurgical procedures, shunt devices, or head trauma; and (2) hematogenous meningitis, sometimes in the context of infective endocarditis.<sup>529</sup>

Postoperative and spontaneous *S. aureus* meningitis are two different clinical syndromes. *Postoperative meningitis* usually appears as hospital-acquired infection in young people, and most cases are associated with ventricular shunt devices,<sup>530</sup> recent neurosurgery, or cerebrospinal fluid leak. On the other hand, *spontaneous meningitis* is a community-acquired infection associated with a variety of clinical sources (primary bacteremia, infective endocarditis, osteomyelitis) that mainly affects older patients with severe underlying conditions.<sup>529</sup> The mortality rate associated with *S. aureus* meningitis is high, around 50%; the mortality rate of spontaneous meningitis is usually higher than that of postoperative meningitis.<sup>530-532</sup>

### Spinal Epidural Abscess

Spinal epidural abscess is a rare infection of the central nervous system, usually occurring after operation or trauma, that can be associated with damage to the spinal cord and nerve roots. *S. aureus* is the leading bacterial pathogen causing spinal epidural abscess, accounting for about two-thirds of cases. Classically, patients have back pain, fever, and neurologic signs.<sup>533</sup> In general, surgical decompression and a long course of intravenous antibiotics are required in order to achieve a successful outcome.

## Pulmonary Infections

### Epidemiology

*S. aureus* is responsible for 2% to 5% of CAP for which hospitalization is required,<sup>534,535</sup> but for 20% to 30% of hospital-acquired pneumonia (HAP).<sup>409</sup> The prevalence of *S. aureus* CAP varies widely by country and continent.<sup>536</sup> Although *S. aureus* CAP cases may cluster with influenza cases, *S. aureus* CAP occurs year round. In recent studies, patients with *S. aureus* CAP had similar demographic characteristics, comorbidities, and presenting signs and symptoms compared with those with non-*S. aureus* CAP.<sup>534</sup> Mortality is high (10%–15%) compared with pneumococcal CAP (about 5%),<sup>534</sup> and is often associated with a more severe course.<sup>535</sup>

In the hospital, *S. aureus* is becoming the pathogen most frequently responsible for nosocomial pneumonia. Its frequency increased from 13% of the cases between 1981 and 1986 to 25% between 2011 and 2014,<sup>409</sup> with a proportion of MRSA that peaked at 55% during the last decade.

### Clinical Spectrum

The clinical manifestations of *S. aureus* pneumonia are frequently indistinguishable from those of pneumonia caused by other pathogens,<sup>534</sup> although the pneumonia caused by *S. aureus* is typically a necrotizing infection with rapid progression to tissue destruction and cavitation. The infection may result either from airborne contamination or aspiration or from hematogenous seeding during bacteremia or right-sided endocarditis. In both cases, the pulmonary infection can lead to local complications, such as abscesses and pleural empyema.

Pneumonia in young, previously healthy adults with a preceding influenza-like illness characterized by severe respiratory symptoms, hemoptysis, high fever, leukopenia, very high C-reactive protein level (>400 mg/L), hypotension, and a chest radiograph that shows multilobar cavitating alveolar infiltrates should lead one to suspect CA-MRSA infection.<sup>411</sup> The fatality rate of CA-MRSA necrotizing pneumonia can be as high as 60%.<sup>537</sup> Airway bleeding, erythroderma, and leukopenia are strong predictors of mortality.<sup>537</sup> Fig. 194.8 illustrates a case of acute

hemorrhagic pneumonia from PVL toxin-secreting *S. aureus* associated with influenza A infection in a 20-year-old patient (see “[Killing Leukocytes](#)” section earlier).

*S. aureus* remains a common cause of pleural empyema and accounts for about one-fifth of the cases.<sup>538</sup> Acute empyema usually arises by means of direct extension from *S. aureus* pneumonia or lung abscess. It is also often seen as a complication of thoracic surgery. Radiologic findings on CT scan or ultrasound scan confirm the clinical suspicion. Demonstration of a pleural air-fluid level in the absence of a previous thoracocentesis suggests a bronchopleural fistula, another feared complication of *S. aureus* infection. [Fig. 194.19](#) illustrates the case of multiple lung abscesses and pleural effusion in a young patient with right-sided endocarditis.

### Therapy

Rapid institution of appropriate antibiotic therapy is essential. Delay is associated with poor outcome. Because the rapid determination of the etiology of severe pneumonia is possible only in a limited number of cases, initial broad-spectrum antibiotic therapy that treats MRSA and other nosocomial pathogens should be instituted early in the cases of health care-associated pneumonia. However, deescalation therapy should occur whenever possible; in particular, a switch to a more rapidly bactericidal β-lactam agent should be done whenever possible according to susceptibility testing. Because of the low prevalence of MRSA in CAP,<sup>534,536</sup> routine empirical anti-MRSA antibiotics are not recommended in the initial treatment of CAP.<sup>539</sup>

For years, vancomycin was the only treatment for MRSA pneumonia, but the cure rate was disappointing. This led to the recommendation to increase trough concentrations of vancomycin in the serum to 15–20 µg/mL for severe infections. Linezolid is an alternative to vancomycin for the treatment of MRSA pneumonia. Clinical outcomes of *S. aureus* HAP and VAP treated with linezolid therapy were found to be significantly better than in those treated with vancomycin in retrospective subgroups analysis. However, these results were not confirmed in a randomized trial. Thus it is still unclear whether one drug is better than the other. For patients with pneumonia caused by MRSA strains with vancomycin MICs of 1.0 mg/mL or more who require concomitant nephrotoxic therapy or who have preexisting renal failure, linezolid (600 mg IV, q12h, with possible later switch to oral therapy) is advised.<sup>540</sup>

The duration of treatment of *S. aureus* pneumonia is determined by the general picture of the disease. The less complicated cases of CAP without overt tissue destruction and without associated deep-seated infections respond to appropriate antibiotic therapy in 10 to 15 days. With the exception of MRSA pneumonia, 7 to 8 days of treatment is sufficient for most patients with HAP or VAP without bacteremia. In the case of surgical drainage of empyema, the treatment duration is adjusted according to cultures and persistence of the pleural effusion. In the case of right-sided endocarditis, therapy is prolonged to 4 weeks according to standard recommendations for endocarditis treatment (see previous discussion).

### Osteoarticular Infections

*S. aureus* is the leading cause of bone and joint infections in both children and adults.<sup>541</sup> The prevalence of those infections is increasing, mainly because of a longer life expectancy, the rise in chronic diseases such as diabetes mellitus, and the increasing use of bone fixation devices and prosthetic joints in the elderly.<sup>542</sup>

### Osteomyelitis

#### Epidemiology

Osteomyelitis has been described since antiquity. Its epidemiology has, however, dramatically changed over the last decades, in parallel with the increased use of medical devices in orthopedic surgery; the higher incidence of chronic disease, especially diabetes mellitus; and the emergence of resistant bacterial strains (e.g., MRSA and CA-MRSA).<sup>543</sup> In parallel, immunization programs led to the disappearance of causative organisms such as *Haemophilus influenzae*, and improved identification methods revealed new causative microorganisms (i.e., *Kingella kingae*). Nevertheless, *S. aureus* continues to be the leading organism, isolated in 30% to 60% of cases ([Table 194.11](#)).<sup>486,544</sup>

**TABLE 194.11 Frequency of Osteomyelitis Caused by Various Microorganisms**

Microorganisms	Frequency of Osteomyelitis (%)			
	Blyth et al. <sup>547</sup>	Tice et al. <sup>557</sup>	Kremers et al. <sup>542</sup>	Murillo et al. <sup>543</sup>
<i>Staphylococcus aureus</i>	65	ND	44	59
Coagulase-negative staphylococci	5	13.9	17	3
Non-group D streptococci	30	13.7	16	17
<i>Pseudomonas aeruginosa</i>	ND	4.4	7	2
Other	0	13.8	16	19
Total	100	100	100	100
Demographics				
No. of patients or episodes	20	454	760	618
Age (yr; median)	0.1–12 (5.4)	6–92 (51)	52 +/- 25	53–77 (65)

ND, Not determined.

The mortality of osteomyelitis has markedly decreased since the introduction of antibacterial therapy. In one pediatric case review, the mortality rate decreased from more than 30% before the introduction of sulfa derivatives (years 1936 through 1940) to approximately 13% afterward (from 1941 to 1945).<sup>545</sup> The mortality rate from osteomyelitis continued to decline with modern antibiotics and is now stabilizing at around 5%.<sup>546</sup>

The reported incidence of osteomyelitis has risen from around 3 cases per 100,000 population per year in 1997<sup>547</sup> to 24.4 cases per 100,000 population per year in recent population-based studies.<sup>542</sup> Whereas the incidence of osteomyelitis is increasing in adults, it has been declining in children younger than 13 years; in the area of the Greater Glasgow Board Health Center (United Kingdom), the incidence rate of osteomyelitis declined by about three times over the last 30 years and by two times during the 1990s.<sup>547</sup> Some studies reported, however, an increased incidence in children related to CA-MRSA.<sup>548</sup>

The incidence of osteomyelitis varies with age and the presence of underlying risk factors such as male sex (men are affected twice as often as women) or diabetes. Other risk factors include individuals with an increased risk of bacteremia, such as patients undergoing hemodialysis or intravenous drug users, and immunosuppression.

#### Pathogenesis

Lew and Waldvogel differentiated three different groups of osteomyelitis etiologies: (1) hematogenous osteomyelitis occurring mostly in children; (2) osteomyelitis due to local spread from a contiguous source after trauma or surgery; and (3) secondary osteomyelitis in patients with vascular insufficiency or concomitant neuropathy, with most of the cases following a foot soft tissue infection.<sup>549</sup> Some authors propose the addition of a fourth etiologic group of osteomyelitis occurring in special hosts with an increased risk for bone infection, including intravenous drug users, individuals with sickle cell anemia, and patients with Gaucher disease.

Healthy bone is generally highly resistant to infection. The two ways by which bacteria can infect the bones are: 1, hematogenous seeding; and 2, contiguous contamination. Both have epidemiologic and pathogenic correlates. Bone infection requires certain predisposing circumstances. In children with hematogenous osteomyelitis, the disease is usually located at the distal end of the long bone metaphysis, including the humerus, femur, and tibia.<sup>548</sup> The nature of the blood flow close to the growing plate may be responsible. Terminal arterioles followed by stagnant blood in the venous sinusoids may facilitate the settlement of

blood-borne staphylococci. A similar model may apply for vertebral osteomyelitis (Fig. 194.20A), wherein blood flow at the vertebral interface is somewhat similar. In addition, microtrauma (or macrotrauma) to bone may facilitate infection by affecting the local blood supply or exposing host matrix proteins to which staphylococci can adhere.

On the bacterial side, *S. aureus* is equipped with several surface adhesins or MSCRAMMs (see Tables 194.1 and 194.3, and “[The Journey to Invasive Disease](#)” earlier), including collagen-binding protein and sialoprotein-binding protein, which were shown to promote experimental osteoarticular infection. After local settlement, secreted proteases, such as aureolysin, and toxins, such as alpha-type PSMs,<sup>550</sup> promote profound alteration in bone remodeling, tissue destruction, and invasion, as indicated by a decreased virulence of *sae*, *sar*, and *agr* mutants that are affected in secretion of virulence factors.<sup>551,552</sup> The combined effect of both *S. aureus* factors and immune cell-mediated production of oxygen radicals and cytokines results in local necrosis and abscess formation. If adequate

antibacterial therapy is given, the nascent abscess can heal totally. Alternatively, bone remodeling and necrosis can extend and circumscribe devitalized bone fragments, or sequestra, floating in the abscess cavity (see Fig. 194.20B). The formation of necrosis and sequestra exemplifies the evolution of acute osteomyelitis to the chronic form (see subsequent discussion) and requires the combination of antibiotics and surgical débridement and sequestrectomy for successful treatment.

### Clinical Features

Osteomyelitis can be classified according to pathogenesis, to localization, to the presence of an implant, or to anatomy and comorbidity. It is conventionally divided into acute and chronic disease. Acute osteomyelitis is defined as a first episode that responds to medical treatment within 6 weeks. It is usually hematogenous and predominant in children and elderly patients. Symptoms are those of an acute septic syndrome with chills, high fever, malaise, and local pain and swelling. Blood cultures are positive in about 50% of cases, and blood or tissue cultures in 65% of cases.

Chronic osteomyelitis is considered in all other situations, including the relapse of previously treated or untreated disease and infection arising by contiguity. The process can evolve over months or even years and is characterized by low-grade inflammation, the presence of necrosis, sequestra, pus, fistula, and recurrences.<sup>549</sup>

Aside from open-wound fractures, contiguous osteomyelitis involves diabetes-related and unrelated vascular diseases and prosthesis-related osteomyelitis. Diabetes-related osteomyelitis and vascular-related osteomyelitis principally involve the feet. They complicate chronic ulcers, which may be paradoxically painless because of associated neuritis. The ulcerative lesion should be explored gently, but in depth, with a surgical probe. If the probe encounters the bone surface, osteomyelitis is present.<sup>553</sup> Other investigations involve radiology and surgical biopsy. Cultures of deep tissues and bone biopsy are mandatory for microbiologic diagnosis. Cultures of surface swabs and fistula fluids mostly yield skin contaminants but not the responsible pathogen.

### Diagnosis

Diagnosis of osteomyelitis integrates clinical signs, radiology, and microbiology. However, clinical signs may be scarce in chronic infection. Imaging techniques are of primary importance, and dominated now by MRI because it is highly sensitive and provides information on both solid and soft tissues. Nuclear studies (bone scans) are performed only if metal hardware precludes MRI or CT.

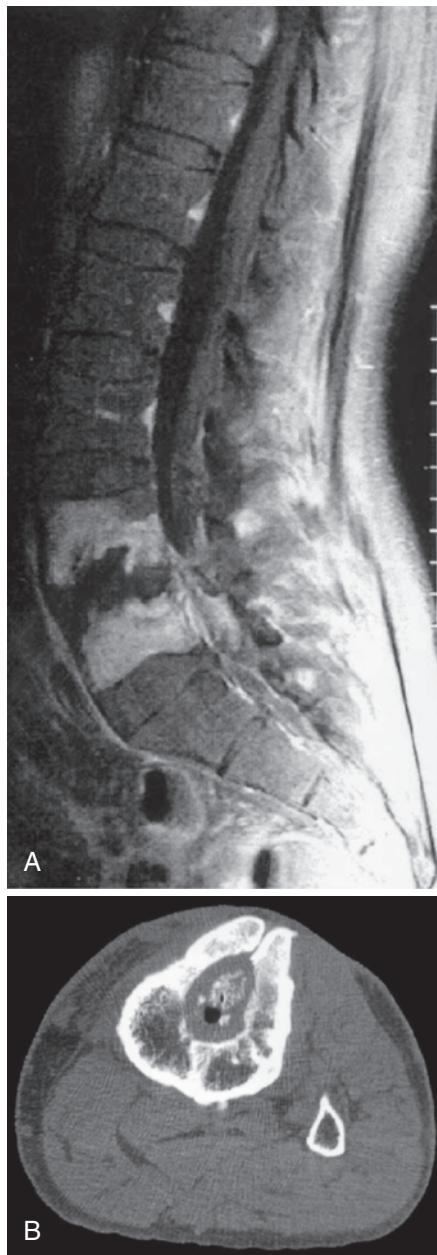
Microbiologic cultures are indispensable to guide therapy. Whenever possible, both blood cultures and tissue biopsies should be performed. Important to note, chronic infection may be associated with persistent forms of *S. aureus*, such as SCVs (small colony variants), especially if aminoglycosides have been used in conjunction with osteosynthetic material (see earlier “[Culture and Identification](#)” section).<sup>554</sup> The diagnostic laboratory should be made aware of this possibility so appropriate measures may be taken.

### Therapy

Rapid institution of antibiotic therapy is mandatory to prevent bone necrosis and the passage of acute osteomyelitis to more problematic chronic disease. The duration of drug treatment in most studies is 4 and 6 weeks but may vary up to 10 weeks or more in complicated situations. A trial suggested that 6 weeks of treatment is noninferior to 12 weeks.<sup>430</sup> Empirical treatment of osteomyelitis should include adequate coverage of MRSA in areas where CA-MRSA is endemic. Adequate representative cultures should be obtained to ensure the early identification of MRSA.

The classic regimens are as follows: (1) for penicillin-susceptible *S. aureus*, intravenous penicillin G, 4 millions units IV q6h; (2) for penicillin-resistant *S. aureus*, intravenous nafcillin, oxacillin, or, outside the United States, flucloxacillin, 2 g IV q6h; and (3) for MRSA, intravenous vancomycin, 1 g IV q12h (followed a loading dose as proposed in “[Therapeutic Monitoring of Vancomycin in Adult Patients](#)”). Clindamycin might be a good alternative to vancomycin in the presence of susceptible CA-MRSA (see Chapter 104).

In children with hematogenous *S. aureus* osteomyelitis, a relatively short course (4–7 days) of intravenous treatment is often followed by oral therapy. A series comparing 131 children showed equivalence



**FIG. 194.20 Osteomyelitis.** (A) T1-weighted magnetic resonance image of L4-L5 lumbar osteomyelitis after injection of gadolinium contrast medium. (B) Chronic osteomyelitis of left tibia. A vast necrotic cavern containing sequestrum surrounded by air can be seen. A fistula with drainage to the skin is also present. (Courtesy Dr. U. Flückiger, Hirslanden Klinik, Aarau, Switzerland.)

between short (total of 20 days) and long (total of 30 days) treatment durations.<sup>555</sup> Intravenous treatment (median 3.7 days) was followed by oral amoxicillin, first-generation cephalosporin, or clindamycin for the remaining period. Oral treatment is currently not recommended in adults, although newer quinolones may be appropriate because of their high bioavailability. However, outpatient therapy is also increasingly used. Although controversial, some workers use ceftriaxone (2 g once a day), which was shown to be effective against *S. aureus* osteomyelitis in two studies.<sup>556,557</sup>

Finally, osteomyelitis from multiresistant MRSA remains difficult to treat. Although vancomycin is still recommended as first-line therapy, daptomycin and linezolid are good alternatives.<sup>558</sup>

### **Native Joint Septic Arthritis**

Septic arthritis is a disease that usually arises in elderly people and very young children.<sup>546,559</sup> *S. aureus* remains the most frequent cause of septic arthritis in both children and adults.<sup>429,543</sup> Previous joint pathology (e.g., rheumatoid arthritis, osteoarthritis, crystal arthropathy) and diabetes are risk factors. The disease may follow both hematogenous seeding and local trauma and may be of iatrogenic nature in the case of joint puncture or arthroscopy. Postarthroscopic septic arthritis has a prevalence of around 14 cases per 10,000 procedures.<sup>560</sup> Symptoms associated are acute pain and joint swelling. Joint destruction occurs within a few days from both bacterial and host inflammatory factors and probably also ischemic lesions from the increased intraarticular hydrostatic pressure. Therefore, patients with underlying arthritis with acute pain in a single joint should undergo aspiration immediately; the fluid should be examined for cell count, chemistry, and gout crystals (if appropriate), and culture should be performed.

The prognosis of hematogenous arthritis in children is good. In adults, the mortality rate is around 11%<sup>429</sup> and the prognosis is mostly associated with that of the underlying disease (i.e., rheumatoid arthritis or endocarditis or other deep-seated condition responsible for the initial bacteremia). Medical treatment is identical to that of osteomyelitis. Open joint drainage is usually unnecessary, except for hip infections in children, where it may help prevent necrosis of the femoral head.

### **Septic Bursitis**

Septic bursitis is an acute infection that involves the periarticular bursa. It is most often located in pressure areas such as the olecranon and the patella. It manifests as an acute juxtaarticular inflammation. The overlying skin is usually inflamed. Unlike arthritis and osteomyelitis, the underlying bone and joint are usually painless at pressure or mobilization. The portal of entry is likely to be local, such as spread from cellulitis. More than 80% of bursitis is the result of *S. aureus*.<sup>561</sup> Diagnosis is made with puncture and examination of the bursa fluid. Important to note, septic bursitis may be at the origin of both local and distant septic complication. Thus, careful clinical evaluation is mandatory. The prognosis is good but requires 2 to 3 weeks of appropriate antibiotic therapy. Repeated aspiration of the bursa is preferable to incision and drainage for patient with persistent swelling and pain. Patients with complicated cases may require hospitalization and intravenous treatment. Surgical excision of the bursa may be considered in the case of recurrences, once inflammation has subsided.

### **Prosthetic Joint Infections**

With the use of perioperative antimicrobial prophylaxis and laminar airflow operating rooms, prosthetic joint infections are fairly rare, with an estimated incidence rate of 1.5 infections per 1000 joint-years.<sup>431</sup> The infection rate after primary joint replacement is usually less than 1% in hip and shoulder prostheses, less than 2% in knee prostheses, and less than 9% in elbow prostheses.<sup>562</sup> Sixty percent to 70% of infections occur during the first 2 years after surgery. Infection rates after surgical revision are usually considerably higher.

Implant-associated infections are typically caused by microorganisms that grow in structures known as biofilms. *S. aureus* and CoNS are the main causative organisms.<sup>431,562</sup> Infections within the 12 first weeks after implantation are considered early, or acute; infections that occur from 12 weeks to 24 months after implantation are considered delayed, or low-grade, infections; and those that occur after 24 months are considered late, or chronic.<sup>562</sup>

*S. aureus* is mostly responsible for early infection.<sup>431</sup> As for prosthetic heart valves, the organisms usually originate from the skin and are likely to be introduced at the time of operation. Although early symptoms may be acute, patients with low-grade or chronic infection may have few general symptoms, and the clinical signs may become centered around local pain and loosening of the prosthesis. Prosthetic joints remain susceptible to hematogenous seeding during their entire lifetime. The overall risk for prosthetic joint infection after *S. aureus* bacteraemia is high, ranging from 30% to 40%.<sup>563,564</sup>

Blood culture results are often negative. Cultures of the fluid from the artificial joint are critical, preferably with broth culture, but can have negative results as well. Molecular diagnosis with PCR should be considered (see “Culture and Identification” earlier). If the prosthesis is surgically removed, multiple microbiologic samples should be taken at various sites of the prosthesis and bone cavity because bacteria may remain clustered in circumscribed areas.

Successful treatment combines surgical intervention and antibiotics. The suggested antibiotic duration is 3 months for hip prostheses and 6 months for knee prostheses. Intravenous therapy should be administered for the first 2 to 4 weeks.<sup>431</sup> The addition of rifampin to a conventional antistaphylococcal regimen appeared clearly useful in patients with prosthetic joint infections. Rifampin has an excellent activity on slow-growing and adherent staphylococci. In general, retention of the implant should not be attempted. Débridement with retention is an option only for patients with early postoperative or acute hematogenous infection, if duration of clinical signs and symptoms is less than 3 weeks, if the implant is stable, and if effective therapy against biofilm microorganisms is available. The prerequisites for one-stage exchange are satisfactory condition of soft tissue, the absence of severe coexisting illnesses, and the absence of difficult-to-treat bacteria. In all other cases, a two-stage exchange is preferred, with a 2-week to 4-week interval between procedures (see Chapter 105).<sup>431,565</sup>

### **Pyomyositis**

Primary pyomyositis, also called tropical myositis, infective myositis, pyogenic myositis, and myositis purulenta tropica, is a rare subacute purulent infection of skeletal muscles. It does not follow contiguous contamination and is most probably of hematogenous origin. The rarity of the disease is attributed to the resistance of muscles to infection. A history of muscle trauma is often reported.

Pyomyositis is frequently seen in Africa and the South Pacific, but it is rare in the Northern Hemisphere. Hence, it could be related to particular local conditions or bacterial properties. In a review of 676 cases, the disease occurred in all age groups. However, it was about twice as frequent in children and adults younger than 30 years than in older adults, and males were predominantly affected.<sup>566</sup> Any muscle may be involved, but the quadriceps and iliopsoas muscles were most often implicated, in 26% and 14%, respectively. *S. aureus* was the etiologic agent in about 80% of cases. In a series from the Amazon Basin of Peru, 11 of 12 patients carried the Panton-Valentine toxin suggesting that PVL might be involved (see “Killing Leukocytes” section earlier).

Clinical symptoms evolve in three stages. They first start with the insidious onset of dull cramping and low-grade fever, general malaise, and muscle aches. Because only the aponeurosis is innervated, overt muscle pain may be delayed for 1 or 2 weeks, before frank abscess formation. Second, the formation of muscle abscess becomes symptomatic, with pain, muscle swelling, tenderness, and sepsis. Most patients are seen at this stage. If left untreated, the disease evolves into the third stage, with muscle destruction, local extension with osteomyelitis, or osteoarthritis, septicemia, and distant dissemination. Diagnosis involves radiographic imaging (CT and MRI) and bacteriologic diagnosis with blood cultures and muscle puncture. Treatment is essentially based on antibiotic therapy. Treatment duration is a matter of debate. Parenteral treatment is often recommended for 7 to 14 days, followed by oral treatment for up to 6 weeks. The prognosis before stage 3 is usually excellent.

### **ACKNOWLEDGMENT**

This work was supported by Swiss National Science Foundation grants CR31I3\_166124 and 320030\_176216 (to YA Que) and 310030\_143650 (to P. Moreillon) on staphylococcal disease and new phage therapy.

**UPDATE****Combination Therapy for MRSA Bacteremia****John E. Bennett, MD**

July 1, 2020

MRSA bacteremia is associated with a mortality of greater than 20%, prompting interest in possibly improved outcomes with combination therapy. Adding a second antimicrobial to vancomycin or daptomycin has been studied with cef-taroline, another  $\beta$ -lactam or rifampin, with no convincingly positive outcome for any of the combinations.<sup>1,2</sup> A randomized open trial, published in 2020, has provided some useful guidance by concluding that adding a week of flucloxacillin or cloxacillin to vancomycin therapy did not improve outcome of MRSA bacteremia.<sup>3</sup> The primary endpoint at 90 days was a composite of mortality, persistent bacteremia at day 5, microbiologic relapse or microbiologic treatment failure. The study was done in 352 hospitalized adults in Australia, New Zealand, Singapore, and Israel. Daptomycin was used in 3.7% instead of vancomycin, which was dosed to trough levels of 15–20 mcg/ml. Cefazolin was permitted in patients hypersensitive to flu/cloxacillin or on hemodialysis. Among the secondary outcomes, bacteremia was shorter with the combination but acute kidney injury was more common. Enrollment was ended early because of nephrotoxicity in the combination group. There were not enough patients in subgroups, such as endocarditis, to permit meaningful analysis. At present, no combination therapy can be recommended for MRSA bacteremia.

**References**

1. <https://pubmed.ncbi.nlm.nih.gov/29249276/>.
2. <https://pubmed.ncbi.nlm.nih.gov/31938716/>.
3. <https://pubmed.ncbi.nlm.nih.gov/32044943/>.

## References

1. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med.* 1998;339:520–532.
2. Spaulding AR, Salgado-Pabon W, Kohler PL, et al. Staphylococcal and streptococcal superantigen exotoxins. *Clin Microbiol Rev.* 2013;26:422–447.
3. Ladhan S, Joannou CL, Lochrie DP, et al. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. *Clin Microbiol Rev.* 1999;12:224–242.
4. Lamand V, Dauwalder O, Tristan A, et al. Epidemiological data of staphylococcal scalded skin syndrome in France from 1997 to 2007 and microbiological characteristics of *Staphylococcus aureus* associated strains. *Clin Microbiol Infect.* 2012;18:E514–E521.
5. Brosnahan AJ, Schlievert PM. Gram-positive bacterial superantigen outside-in signaling causes toxic shock syndrome. *FEBS J.* 2011;278:4649–4667.
6. Jarraud S, Peyrat MA, Lim A, et al. egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J Immunol.* 2001;166:669–677.
7. Feng Y, Chen CJ, Su LH, et al. Evolution and pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. *FEMS Microbiol Rev.* 2008;32:23–37.
8. Baba T, Takeuchi F, Kuroda M, et al. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet.* 2002;359:1819–1827.
9. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol.* 2005;3:948–958.
10. Lindsay J, Holden M. Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. *Funct Integr Genomics.* 2006;6:186–201.
11. Gotz F, Bannerman T, Schleifer K-H. The genera *Staphylococcus* and *Macrococcus*. In: Balows A, Truper AG, Dworkin M, et al, eds. *The Prokaryotes*. Vol. 4. 3rd ed. New York: Springer Science+Business Media; 2006:1–75.
12. Kloos WE. *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*. 2nd ed. New York: Springer-Verlag; 1992.
13. Novick RP, Ram G. The floating (pathogenicity) island: a genomic dessert. *Trends Genet.* 2016;32:114–126.
14. Kuroda M, Ohta T, Uchiyama I, et al. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet.* 2001;357:1225–1240.
15. Ogston A. Micrococcus poisoning. *J Anat Physiol.* 1883;17:24–58.
16. Takeuchi F, Watanabe S, Baba T, et al. Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. *J Bacteriol.* 2005;187:7292–7308.
17. Kuroda M, Yamashita A, Hirakawa H, et al. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc Natl Acad Sci USA.* 2005;102:13272–13277.
18. Gill SR, Fouts DE, Archer GL, et al. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *J Bacteriol.* 2005;187:2426–2438.
19. Björansson S, Gomez-Sanz E, Ekstrom K, et al. *Staphylococcus pseudintermedius* can be misdiagnosed as *Staphylococcus aureus* in humans with dog bite wounds. *Eur J Clin Microbiol Infect Dis.* 2015;34:839–844.
20. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev.* 1997;10:505–520.
21. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev.* 1963;27:56–71.
22. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis.* 2005;5:751–762.
23. Senn L, Clerc O, Zanetti G, et al. The stealthy superbug: the role of asymptomatic enteric carriage in maintaining a long-term hospital outbreak of ST228 methicillin-resistant *Staphylococcus aureus*. *MBio.* 2016;7:e02039–02015.
24. von Eiff C, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med.* 2001;344:11–16.
25. Dufour P, Gillet Y, Bes M, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. *Clin Infect Dis.* 2002;35:S19–S24.
26. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med.* 2010;362:9–17.
27. Kuehnert MJ, Kruszon-Moran D, Hill HA, et al. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. *J Infect Dis.* 2006;193:172–179.
28. Compernolle V, Verschraegen G, Claeys G. Combined use of Pastorex Staph-Plus and either of two new chromogenic agars, MRSA ID and CHROMagar MRSA, for detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2007;45:154–158.
29. Walsh TR, Bolmstrom A, Qwarnstrom A, et al. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. *J Clin Microbiol.* 2001;39:2439–2444.
30. Proctor RA, Balwit JM, Vesga O. Variant subpopulations of *Staphylococcus aureus* as cause of persistent and recurrent infections. *Infect Agents Dis.* 1994;3:302–312.
31. Wolter DJ, Emerson JC, McNamara S, et al. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. *Clin Infect Dis.* 2013;57:384–391.
32. Baumert N, von Eiff C, Schaaff F, et al. Physiology and antibiotic susceptibility of *Staphylococcus aureus* small colony variants. *Microb Drug Resist.* 2002;8:253–260.
33. Edwards AM. Phenotype switching is a natural consequence of *Staphylococcus aureus* replication. *J Bacteriol.* 2012;194:5404–5412.
34. Latimer J, Forbes S, McBain AJ. Attenuated virulence and biofilm formation in *Staphylococcus aureus* following sublethal exposure to triclosan. *Antimicrob Agents Chemother.* 2012;56:3092–3100.
35. Chatterjee I, Kriegeskorte A, Fischer A, et al. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. *J Bacteriol.* 2008;190:834–842.
36. Jonsson IM, von Eiff C, Proctor RA, et al. Virulence of a hemB mutant displaying the phenotype of a *Staphylococcus aureus* small colony variant in a murine model of septic arthritis. *Microb Pathog.* 2003;34:73–79.
37. Bates DM, Eiff Cv C, McNamara PJ, et al. *Staphylococcus aureus* menD and hemB mutants are as infective as the parent strains, but the menadione biosynthetic mutant persists within the kidney. *J Infect Dis.* 2003;187:1654–1661.
38. Vaudaux P, Francois P, Bisognano C, et al. Increased expression of clumping factor and fibronectin-binding proteins by hemB mutants of *Staphylococcus aureus* expressing small colony variant phenotypes. *Infect Immun.* 2002;70:5428–5437.
39. Williamson DA, Heffernan H, Nimmo G. Contemporary genomic approaches in the diagnosis and typing of *Staphylococcus aureus*. *Pathology.* 2015;47:270–275.
40. Carretero E, Bardaro M, Russello G, et al. Comparison of the *Staphylococcus* QuickFISH BC test with the tube coagulase test performed on positive blood cultures for evaluation and application in a clinical routine setting. *J Clin Microbiol.* 2013;51:131–135.
41. Laub RR, Knudsen JD. Clinical consequences of using PNA-FISH in Staphylococcal bacteraemia. *Eur J Clin Microbiol Infect Dis.* 2014;33:599–601.
42. Huletsky A, Lebel P, Picard FJ, et al. Identification of methicillin-resistant *Staphylococcus aureus* carriage in less than 1 hour during a hospital surveillance program. *Clin Infect Dis.* 2005;40:976–981.
43. Zhang K, McClure JA, Elsayed S, et al. Novel multiplex PCR assay for simultaneous identification of community-associated methicillin-resistant *Staphylococcus aureus* strains USA300 and USA400 and detection of meCA and Panton-Valentine leukocidin genes, with discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *J Clin Microbiol.* 2008;46:1118–1122.
44. Okolie CE, Wooldridge KG, Turner DP, et al. Development of a new pentaplex real-time PCR assay for the identification of poly-microbial specimens containing *Staphylococcus aureus* and other staphylococci, with simultaneous detection of staphylococcal virulence and methicillin resistance markers. *Mol Cell Probes.* 2015;29:144–150.
45. Harbarth S, Masuet-Aumatell C, Schrenzel J, et al. Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study. *Crit Care.* 2006;10:R25.
46. Mendes RE, Watters AA, Rhomberg PR, et al. Performance of BD max StaphSR for screening of methicillin-resistant *Staphylococcus aureus* isolates among a contemporary and diverse collection from 146 institutions located in nine U.S. census regions: prevalence of meCA dropout mutants. *J Clin Microbiol.* 2016;54:204–207.
47. Ito T, Hiramatsu K, Tomasz A, et al. Guidelines for reporting novel meCA gene homologues. *Antimicrob Agents Chemother.* 2012;56:4997–4999.
48. Garcia-Alvarez L, Holden MT, Lindsay H, et al. Methicillin-resistant *Staphylococcus aureus* with a novel meCA homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis.* 2011;11:595–603.
49. Proulx MK, Palace SG, Gandra S, et al. Reversion from methicillin susceptibility to methicillin resistance in *Staphylococcus aureus* during treatment of bacteraemia. *J Infect Dis.* 2016;213:1041–1048.
50. Sogawa K, Watanabe M, Ishige T, et al. Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* using MALDI-TOF mass spectrometry. *Bicontrol Sci.* 2017;22:163–169.
51. Ruppe E, Cherkaoui A, Lazarevic V, et al. Establishing genotype-to-phenotype relationships in bacteria causing hospital-acquired pneumonia: a prelude to the application of clinical metagenomics. *Antibiotics (Basel).* 2017;6.
52. Styers D, Sheehan DJ, Hogan P, et al. Laboratory-based surveillance of current antimicrobial resistance patterns and trends among *Staphylococcus aureus*: 2005 status in the United States. *Ann Clin Microbiol Antimicrob.* 2006;5:2.
53. Kanjilal S, Sater MRA, Thayer M, et al. Trends in Antibiotic Susceptibility in *Staphylococcus aureus* in Boston, Massachusetts, from 2000 to 2014. *J Clin Microbiol.* 2018;56.
54. Cai Y, Venkatachalam I, Tee NW, et al. Prevalence of healthcare-associated infections and antimicrobial use among adult inpatients in Singapore acute-care hospitals: results from the first national point prevalence survey. *Clin Infect Dis.* 2017;64(suppl\_2):S61–S67.
55. Enright MC, Robinson DA, Randle G, et al. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci USA.* 2002;99:7687–7692.
56. Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis.* 2002;2:180–189.
57. Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol.* 2000;38:1008–1015.
58. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2003;47:3926–3934.
59. Kuhn G, Francioli P, Blanc DS. Double-locus sequence typing using clfB and spa, a fast and simple method for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2007;45:54–62.
60. Rasigade JP, Laurent F, Lina G, et al. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981–2007. *J Infect Dis.* 2010;201:1589–1597.
61. Novick RP, Geisinger E. Quorum sensing in staphylococci. *Annu Rev Genet.* 2008;42:541–564.
62. Pragman AA, Schlievert PM. Virulence regulation in *Staphylococcus aureus*: the need for in vivo analysis of virulence factor regulation. *FEMS Immunol Med Microbiol.* 2004;42:147–154.
63. Balasubramanian D, Harper L, Shopsin B, et al. *Staphylococcus aureus* pathogenesis in diverse host environments. *Pathog Dis.* 2017;75.
64. Novick RP, Ross HF, Projan SJ, et al. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. *EMBO J.* 1993;12:3967–3975.
65. Cheung AL, Bayer AS, Zhang G, et al. Regulation of virulence determinants in vitro and in vivo in *Staphylococcus aureus*. *FEMS Immunol Med Microbiol.* 2004;40:1–9.
66. Gupta RK, Luong TT, Lee CY. RNAlII of the *Staphylococcus aureus* agr system activates global regulator MgrA by stabilizing mRNA. *Proc Natl Acad Sci USA.* 2015;112:14036–14041.
67. Giraudot AT, Cheung AL, Nagel R. The sae locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. *Arch Microbiol.* 1997;168:53–58.
68. Yarwood JM, McCormick JK, Schlievert PM. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus*. *J Bacteriol.* 2001;183:1113–1123.

69. Fournier B, Hooper DC. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. *J Bacteriol.* 2000;182:3955–3964.
70. Berends ET, Horswill AR, Haste NM, et al. Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. *J Innate Immun.* 2010;2:576–586.
71. Luong TT, Dunman PM, Murphy E, et al. Transcription Profiling of the *mgrA* Regulon in *Staphylococcus aureus*. *J Bacteriol.* 2006;188:1899–1910.
72. Said-Salim B, Dunman PM, McAleese FM, et al. Global regulation of *Staphylococcus aureus* genes by *Rot*. *J Bacteriol.* 2003;185:610–619.
73. Chan PF, Foster SJ. The role of environmental factors in the regulation of virulence-determinant expression in *Staphylococcus aureus* 8325-4. *Microbiology*. 1998;144(Pt 9):2469–2479.
74. Bischoff M, Dunman P, Kormanec J, et al. Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. *J Bacteriol.* 2004;186:4085–4099.
75. Entenza JM, Moreillon P, Senn MM, et al. Role of sigmaB in the expression of *Staphylococcus aureus* cell wall adhesins ClfA and FnBpA and contribution to infectivity in a rat model of experimental endocarditis. *Infect Immun.* 2005;73:990–998.
76. Lorenz U, Huttiger C, Schafer T, et al. The alternative sigma factor sigma B of *Staphylococcus aureus* modulates virulence in experimental central venous catheter-related infections. *Microbes Infect.* 2008;10:217–223.
77. Lioliou E, Sharma CM, Caldelari I, et al. Global regulatory functions of the *Staphylococcus aureus* endoribonuclease III in gene expression. *PLoS Genet.* 2012;8:e1002782.
78. Priest NK, Rudkin JK, Feil EJ, et al. From genotype to phenotype: can systems biology be used to predict *Staphylococcus aureus* virulence? *Nat Rev Microbiol.* 2012;10:791–797.
79. Mayville P, Ji G, Beavis R, et al. Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc Natl Acad Sci USA.* 1999;96:1218–1223.
80. Cheung AL, Eberhardt KJ, Chung E, et al. Diminished virulence of a sar-/agr- mutant of *Staphylococcus aureus* in the rabbit model of endocarditis. *J Clin Invest.* 1994;94:1815–1822.
81. Que YA, Haefliger JA, Piroth L, et al. Fibrinogen and fibronectin binding cooperate for valve infection and invasion in *Staphylococcus aureus* experimental endocarditis. *J Exp Med.* 2005;201:1627–1635.
82. Piroth L, Que YA, Widmer E, et al. The fibrinogen- and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein A synergistically promote endothelial invasion and experimental endocarditis. *Infect Immun.* 2008;76:3824–3831.
83. Cheung AL, Nast CC, Bayer AS. Selective activation of sar promoters with the use of green fluorescent protein transcriptional fusions as the detection system in the rabbit endocarditis model. *Infect Immun.* 1998;66:5988–5993.
84. Yarwood JM, McCormick JK, Paustian ML, et al. Repression of the *Staphylococcus aureus* accessory gene regulator in serum and in vivo. *J Bacteriol.* 2002;184:1095–1101.
85. Goerke C, Wolz C. Adaptation of *Staphylococcus aureus* to the cystic fibrosis lung. *Int J Med Microbiol.* 2010;300:520–525.
86. Smyth DS, Kafer JM, Wasserman GA, et al. Nasal carriage as a source of agr-defective *Staphylococcus aureus* bacteremia. *J Infect Dis.* 2012;206:1168–1177.
87. Boles BR, Horswill AR. Agr-mediated dispersal of *Staphylococcus aureus* biofilms. *PLoS Pathog.* 2008;4:e100052.
88. Archer NK, Mazaitis MJ, Costerton JW, et al. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence.* 2011;2:445–459.
89. Ji G, Beavis R, Novick RP. Bacterial interference caused by autoinducing peptide variants. *Science.* 1997;276:2027–2030.
90. Kahl BC, Becker K, Friedrich AW, et al. agr-dependent bacterial interference has no impact on long-term colonization of *Staphylococcus aureus* during persistent airway infection of cystic fibrosis patients. *J Clin Microbiol.* 2003;41:5199–5201.
91. Dufour P, Jarraud S, Vandenesch F, et al. High genetic variability of the agr locus in *Staphylococcus* species. *J Bacteriol.* 2002;184:1180–1186.
92. Weidenmaier C, Kokai-Kun JF, Kristian SA, et al. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat Med.* 2004;10:243–245.
93. O'Brien LM, Walsh EJ, Massey RC, et al. *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. *Cell Microbiol.* 2002;4:759–770.
94. Roche FM, Meehan M, Foster TJ. The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. *Microbiology*. 2003;149(Pt 10):2759–2767.
95. Corrigan RM, Majlovic H, Foster TJ. Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. *BMC Microbiol.* 2009;9:22.
96. Patti JM, Allen BL, McGavin MJ, et al. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu Rev Microbiol.* 1994;48:585–617.
97. Roche FM, Massey R, Peacock SJ, et al. Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. *Microbiology*. 2003;149(Pt 3):643–654.
98. Mazmanian SK, Liu G, Ton-That H, et al. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science*. 1999;285:760–763.
99. Bradshaw WJ, Davies AH, Chambers CJ, et al. Molecular features of the sortase enzyme family. *FEBS J.* 2015;282:2097–2114.
100. Askarian F, Ajayi C, Hanssen AM, et al. The interaction between *Staphylococcus aureus* SdrD and desmoglein 1 is important for adhesion to host cells. *Sci Rep.* 2016;6.
101. Geoghegan JA, Corrigan RM, Gruszka DT, et al. Role of surface protein SasG in biofilm formation by *Staphylococcus aureus*. *J Bacteriol.* 2010;192:5663–5673.
102. Mazmanian SK, Skaar EP, Gaspar AH, et al. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science*. 2003;299:906–909.
103. Clement S, Vaudaux P, Francois P, et al. Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. *J Infect Dis.* 2005;192:1023–1028.
104. Nouwen J, Boelens H, van Belkum A, et al. Human factor in *Staphylococcus aureus* nasal carriage. *Infect Immun.* 2004;72:6685–6688.
105. Grothe C, Taminato M, Belasco A, et al. Prophylactic treatment of chronic renal disease in patients undergoing peritoneal dialysis and colonized by *Staphylococcus aureus*: a systematic review and meta-analysis. *BMC Nephrol.* 2016;17:115.
106. Chirouze C, Alla F, Fowler VG Jr, et al. Impact of early valve surgery on outcome of *Staphylococcus aureus* prosthetic valve infective endocarditis: analysis in the International Collaboration of Endocarditis-Prospective Cohort Study. *Clin Infect Dis.* 2015;60:741–749.
107. McDevitt D, Francois P, Vaudaux P, et al. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol Microbiol.* 1994;11:237–248.
108. Massey RC, Kantanou MN, Fowler T, et al. Fibronectin-binding protein A of *Staphylococcus aureus* has multiple, substituting, binding regions that mediate adherence to fibronectin and invasion of endothelial cells. *Cell Microbiol.* 2001;3:839–851.
109. Massey RC, Dissanayake SR, Cameron B, et al. Functional blocking of *Staphylococcus aureus* adhesins following growth in ex vivo media. *Infect Immun.* 2002;70:5339–5345.
110. Kroh HK, Panizzi P, Bock PE, Von Willebrand factor-binding protein is a hysteretic conformational activator of prothrombin. *Proc Natl Acad Sci USA.* 2009;106:7786–7791.
111. Rooijakkers SH, Ruyken M, van Roon J, et al. Early expression of SCIN and CHIPS drives instant immune evasion by *Staphylococcus aureus*. *Cell Microbiol.* 2006;8:1282–1293.
112. Peeters M, Vanassche T, Liesenborghs L, et al. Plasminogen activation by staphylokinase enhances local spreading of *S. aureus* in skin infections. *BMC Microbiol.* 2014;14:310.
113. van Wamel WJ, Rooijakkers SH, Ruyken M, et al. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on a beta-hemolysin-converting bacteriophages. *J Bacteriol.* 2006;188:1310–1315.
114. Resch G, Francois P, Morisset D, et al. Human-to-bovine jump of *Staphylococcus aureus* CC8 is associated with the loss of a beta-hemolysin converting prophage and the acquisition of a new staphylococcal cassette chromosome. *PLoS ONE.* 2013;8:e58187.
115. Patti JM, Bremell T, Krajewska-Pietrasik D, et al. The *Staphylococcus aureus* collagen adhesin is a virulence determinant in experimental septic arthritis. *Infect Immun.* 1994;62:152–161.
116. Xu Y, Rivas JM, Brown EL, et al. Virulence potential of the staphylococcal adhesin CNA in experimental arthritis is determined by its affinity for collagen. *J Infect Dis.* 2004;189:2323–2333.
117. Liesenborghs L, Verhamme P, Vanassche T. *Staphylococcus aureus*, master manipulator of the human hemostatic system. *J Thromb Haemost.* 2018;16:441–454.
118. Loughman A, Fitzgerald JR, Brennan MP, et al. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. *Mol Microbiol.* 2005;57:804–818.
119. Ruggeri ZM, Ware J, von Willebrand factor. *FASEB J.* 1993;7:308–316.
120. Claes J, Liesenborghs L, Peeters M, et al. Clumping factor A, von Willebrand factor-binding protein and von Willebrand factor anchor *Staphylococcus aureus* to the vessel wall. *J Thromb Haemost.* 2017;15:1009–1019.
121. Veloso TR, Que YA, Chaouch A, et al. Prophylaxis of experimental endocarditis with antiplatelet and antithrombin agents: a role for long-term prevention of infective endocarditis in humans? *J Infect Dis.* 2015;211:72–79.
122. Claes J, Vanassche T, Peeters M, et al. Adhesion of *Staphylococcus aureus* to the vessel wall under flow is mediated by von Willebrand factor-binding protein. *Blood.* 2014;124:1669–1676.
123. Kuperwasser LI, Yeaman MR, Shapiro SM, et al. In vitro susceptibility to thrombin-induced platelet microbicidal protein is associated with reduced disease progression and complication rates in experimental *Staphylococcus aureus* endocarditis: microbiological, histopathologic, and echocardiographic analyses. *Circulation.* 2002;105:746–752.
124. Bayer AS, Prasad R, Chandra J, et al. In vitro resistance of *Staphylococcus aureus* to thrombin-induced platelet microbicidal protein is associated with alterations in cytoplasmic membrane fluidity. *Infect Immun.* 2000;68:3548–3553.
125. Bayer AS, Cheng D, Yeaman MR, et al. In vitro resistance to thrombin-induced platelet microbicidal protein among clinical bacteremic isolates of *Staphylococcus aureus* correlates with an endovascular infectious source. *Antimicrob Agents Chemother.* 1998;42:3169–3172.
126. Resch A, Rosenstein R, Nerz C, et al. Differential gene expression profiling of *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. *Appl Environ Microbiol.* 2005;71:2663–2676.
127. Kim HK, Thammavongsawat V, Schneewind O, et al. Recurrent infections and immune evasion strategies of *Staphylococcus aureus*. *Curr Opin Microbiol.* 2012;15:92–99.
128. Guerra FE, Borgogna TR, Patel DM, et al. Epic immune battles of history: neutrophils vs. *Staphylococcus aureus*. *Front Cell Infect Microbiol.* 2017;7:286.
129. Wolf AJ, Underhill DM. Peptidoglycan recognition by the innate immune system. *Nat Rev Immunol.* 2018.
130. Chavakis T, Hussain M, Kanse SM, et al. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat Med.* 2002;8:687–693.
131. Higgins J, Loughman A, van Kessel KP, et al. Clumping factor A of *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. *FEMS Microbiol Lett.* 2006;258:290–296.
132. Thiel M, Caldwell CC, Sitkovsky MV. The critical role of adenosine A2A receptors in downregulation of inflammation and immunity in the pathogenesis of infectious diseases. *Microbes Infect.* 2003;5:515–526.
133. Thammavongsawat V, Kern JW, Missiakas DM, et al. *Staphylococcus aureus* synthesizes adenosine to escape host immune responses. *J Exp Med.* 2009;206:2417–2427.
134. Smith EJ, Visai L, Kerrigan SW, et al. The Sbi protein is a multifunctional immune evasion factor of *Staphylococcus aureus*. *Infect Immun.* 2011;79:3801–3809.
135. Zipfel PF. Complement and immune defense: from innate immunity to human diseases. *Immunol Lett.* 2009;126:1–7.
136. Lee LY, Hock M, Haviland D, et al. Inhibition of complement activation by a secreted *Staphylococcus aureus* protein. *J Infect Dis.* 2004;190:571–579.
137. Sharp JA, Echague CG, Hair PS, et al. *Staphylococcus aureus* surface protein SdrE binds complement regulator factor H as an immune evasion tactic. *PLoS ONE.* 2012;7:e38407.
138. Beavers WN, Skaar EP. Neutrophil-generated oxidative stress and protein damage in *Staphylococcus aureus*. *Pathog Dis.* 2016;74.
139. Reichardt V, Reichardt U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science.* 2004;303:1532–1535.
140. Lehrer RI, Ganz T. Antimicrobial peptides in mammalian and insect host defence. *Curr Opin Immunol.* 1999;11:23–27.
141. Peschel A, Collins LV. Staphylococcal resistance to antimicrobial peptides of mammalian and bacterial origin. *Peptides.* 2001;22:1651–1659.

142. Sass P, Bierbaum G. Native graS mutation supports the susceptibility of *Staphylococcus aureus* strain SG511 to antimicrobial peptides. *Int J Med Microbiol.* 2009;299:313–322.
143. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev.* 2000;13:16–34. Table of contents.
144. Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med.* 2007;13:1510–1514.
145. Bhakdi S, Tranum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. *Microbiol Rev.* 1991;55:733–751.
146. Berube BJ, Bubeck Wardenburg J. *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. *Toxins (Basel).* 2013;5:1140–1166.
147. Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. *Proc Natl Acad Sci USA.* 2010;107:13473–13478.
148. Popov LM, Marceau CD, Starkl PM, et al. The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. *Proc Natl Acad Sci USA.* 2015;112:14337–14342.
149. Towle KM, Lohans CT, Miskolzie M, et al. Solution Structures of Phenol-Soluble Modulins alpha1, alpha3, and beta2, Virulence Factors from *Staphylococcus aureus*. *Biochemistry.* 2016;55:4798–4806.
150. von Eiff C, Friedrich AW, Peters G, et al. Prevalence of genes encoding for members of the staphylococcal leukocidin family among clinical isolates of *Staphylococcus aureus*. *Diagn Microbiol Infect Dis.* 2004;49:157–162.
151. Saeed K, Gould I, Esposito S, et al. Panton-Valentine leukocidin-positive *Staphylococcus aureus*: a position statement from the International Society of Chemotherapy. *Int J Antimicrob Agents.* 2018;51:16–25.
152. Sanchini A, Del Grossi M, Villa L, et al. Typing of Panton-Valentine leukocidin-encoding phages carried by methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* from Italy. *Clin Microbiol Infect.* 2014;20:O840–O846.
153. Moran GJ, Krishnasadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med.* 2006;355:666–674.
154. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis.* 1999;29:1128–1132.
155. Nielsen MM, Witherden DA, Havran WL. gammaMELT cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* 2017;17:733–745.
156. Archer NK, Adappa ND, Palmer JN, et al. Interleukin-17A (IL-17A) and IL-17F are critical for antimicrobial peptide production and clearance of *Staphylococcus aureus* nasal colonization. *Infect Immun.* 2016;84:3575–3583.
157. Cho JS, Pietras EM, Garcia NC, et al. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest.* 2010;120:1762–1773.
158. Tomi NS, Kranke B, Aberer E. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. *J Am Acad Dermatol.* 2005;53:67–72.
159. Banci MJ, Veltrop MH, Bertina RM, et al. Role of phagocytosis in activation of the coagulation system in *Streptococcus sanguis* endocarditis. *Infect Immun.* 1996;64:5166–5170.
160. Patel R. Biofilms and antimicrobial resistance. *Clin Orthop Relat Res.* 2005;437:41–47.
161. Begun J, Gaiani JM, Rohde H, et al. Staphylococcal biofilm expolysaccharide protects against *Caenorhabditis elegans* immune defenses. *PLoS Pathog.* 2007;3:e57.
162. Gerke C, Kraft A, Sussmuth R, et al. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J Biol Chem.* 1998;273:18586–18593.
163. Heilmann C, Schweitzer O, Gerke C, et al. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol Microbiol.* 1996;20:1083–1091.
164. Francois P, Tu Quoc PH, Bisognano C, et al. Lack of biofilm contribution to bacterial colonisation in an experimental model of foreign body infection by *Staphylococcus aureus* and *Staphylococcus epidermidis*. *FEMS Immunol Med Microbiol.* 2003;35:135–140.
165. Periasamy S, Joo HS, Duong AC, et al. How *Staphylococcus aureus* biofilms develop their characteristic structure. *Proc Natl Acad Sci USA.* 2012;109:1281–1286.
166. Von Rittershain GR. Die exfoliative dermatitis jungere senglinge. *Z Kinderheilkd.* 1878;2.
167. Yamaguchi T, Nishifuji K, Sasaki M, et al. Identification of the *Staphylococcus aureus* etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. *Infect Immun.* 2002;70:5835–5845.
168. Ladhami S. Understanding the mechanism of action of the exfoliative toxin of *Staphylococcus aureus*. *FEMS Immunol Med Microbiol.* 2003;39:181–189.
169. Staiman A, Hsu DY, Silverberg JI. Epidemiology of staphylococcal scalded skin syndrome in U.S. children. *Br J Dermatol.* 2017.
170. Becker K, Friedrich AW, Lubritz G, et al. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol.* 2003;41:1434–1439.
171. Nishifuji K, Sugai M, Amagai M. Staphylococcal exfoliative toxins: “molecular scissors” of bacteria that attack the cutaneous defense barrier in mammals. *J Dermatol Sci.* 2008;49:21–31.
172. Nishifuji K, Shimizu A, Ishiko A, et al. Removal of amino-terminal extracellular domains of desmoglein 1 by staphylococcal exfoliative toxin is sufficient to initiate epidermal blister formation. *J Dermatol Sci.* 2010;59:184–191.
173. Lina G, Bohach GA, Nair SP, et al. Standard nomenclature for the superantigens expressed by *Staphylococcus*. *J Infect Dis.* 2004;189:2334–2336.
174. Fraser JD, Profitt T. The bacterial superantigen and superantigen-like proteins. *Immunol Rev.* 2008;225:226–243.
175. Stevens F. The occurrence of *Staphylococcus aureus* infection with a scarlatiniform rash. *JAMA.* 1927;88:1957–1958.
176. Shands KN, Schmid GP, Dan BB, et al. Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *N Engl J Med.* 1980;303:1436–1442.
177. Shupp JW, Jett M, Pontzer CH. Identification of a transcytosis epitope on staphylococcal enterotoxins. *Infect Immun.* 2002;70:2178–2186.
178. Stolz SJ, Davis JP, Vergeront JM, et al. Development of serum antibody to toxic shock toxin among individuals with toxic shock syndrome in Wisconsin. *J Infect Dis.* 1985;151:883–889.
179. Reingold AL, Hargrett NT, Shands KN, et al. Toxic shock syndrome surveillance in the United States, 1980 to 1981. *Ann Intern Med.* 1982;96(6 Pt 2):875–880.
180. McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. *Annu Rev Microbiol.* 2001;52:77–104.
181. Hajjeh RA, Reingold A, Weil A, et al. Toxic shock syndrome in the United States: surveillance update, 1979–1996. *Emerg Infect Dis.* 1999;5:807–810.
182. Adalat S, Dawson T, Hackett SJ, et al. In association with the British Paediatric Surveillance Unit. Toxic shock syndrome and bacterial superantigens: an update. *Arch Dis Child.* 2014;99:1078–1082.
183. Lamagni TL, Neal S, Keshishian C, et al. Severe *Streptococcus pyogenes* infections, United Kingdom, 2003–2004. *Emerg Infect Dis.* 2008;14:202–209.
184. Stevens DL, Bisno AL, Chambers HF, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the infectious diseases society of America. *Clin Infect Dis.* 2014;59:147–159.
185. Stevens DL, Gibbons AE, Bergstrom R, et al. The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *J Infect Dis.* 1988;158:23–28.
186. Shah SS, Hall M, Srivastava R, et al. Intravenous immunoglobulin in children with streptococcal toxic shock syndrome. *Clin Infect Dis.* 2009;49:1369–1376.
187. Kadri SS, Swihart BJ, Bonne SL, et al. Impact of intravenous immunoglobulin on survival in necrotizing fasciitis with vasopressor-dependent shock: a propensity score-matched analysis from 130 US hospitals. *Clin Infect Dis.* 2017;64:877–885.
188. Schwameis M, Roppenser B, Firbas C, et al. Safety, tolerability, and immunogenicity of a recombinant toxic shock syndrome toxin (rTSST)-1 variant vaccine: a randomised, double-blind, adjuvant-controlled, dose escalation first-in-man trial. *Lancet Infect Dis.* 2016;16:1036–1044.
189. Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins (Basel).* 2010;2:1751–1773.
190. Fitzgerald JR, Reid SD, Ruotsalainen E, et al. Genome diversification in *Staphylococcus aureus*: molecular evolution of a highly variable chromosomal region encoding the Staphylococcal exotoxin-like family of proteins. *Infect Immun.* 2003;71:2827–2838.
191. Petersson K, Pettersson H, Skartved NJ, et al. Staphylococcal enterotoxin H induces V alpha-specific expansion of T cells. *J Immunol.* 2003;170:4148–4154.
192. Yarwood JM, Leung DY, Schlievert PM. Evidence for the involvement of bacterial superantigens in psoriasis, atopic dermatitis, and Kawasaki syndrome. *FEMS Microbiol Lett.* 2000;192:1–7.
193. Semic Jusufagic A, Bachert C, Gevaert P, et al. *Staphylococcus aureus* sensitization and allergic disease in early childhood: population-based birth cohort study. *J Allergy Clin Immunol.* 2007;119:930–936.
194. Matsubara K, Fukaya T. The role of superantigens of group A *Streptococcus* and *Staphylococcus aureus* in Kawasaki disease. *Curr Opin Infect Dis.* 2007;20:298–303.
195. Feil EJ, Cooper JE, Grundmann H, et al. How clonal is *Staphylococcus aureus*? *J Bacteriol.* 2003;185:3307–3316.
196. Robinson DA, Monk AB, Cooper JE, et al. Evolutionary genetics of the accessory gene regulator (agr) locus in *Staphylococcus aureus*. *J Bacteriol.* 2005;187:8312–8321.
197. Wright JS 3rd, Traber KE, Corrigan R, et al. The agr radiation: an early event in the evolution of staphylococci. *J Bacteriol.* 2005;187:5585–5594.
198. Kang CK, Cho JE, Choi YJ, et al. agr dysfunction affects staphylococcal cassette chromosome mec type-dependent clinical outcomes in methicillin-resistant *Staphylococcus aureus* bacteraemia. *Antimicrob Agents Chemother.* 2015;59:3125–3132.
199. McAdam PR, Templeton KE, Edwards GF, et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci USA.* 2012;109:9107–9112.
200. Fitzgerald JR. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *Trends Microbiol.* 2012;20:192–198.
201. Drougka E, Foka A, Koutinas CK, et al. Interspecies spread of *Staphylococcus aureus* clones among companion animals and human close contacts in a veterinary teaching hospital. A cross-sectional study in Greece. *Prev Vet Med.* 2016;126:190–198.
202. Schwaber MJ, Navon-Venezia S, Masarwa S, et al. Clonal transmission of a rare methicillin-resistant *Staphylococcus aureus* genotype between horses and staff at a veterinary teaching hospital. *Vet Microbiol.* 2013;162:907–911.
203. Steinman A, Masarwa S, Tirosh-Levy S, et al. Methicillin-Resistant *Staphylococcus aureus* spa Type t002 Outbreak in Horses and Staff at a Veterinary Teaching Hospital after Its Presumed Introduction by a Veterinarian. *J Clin Microbiol.* 2015;53:2827–2831.
204. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis.* 2005;11:711–714.
205. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio.* 2012;3:e00305–e00311.
206. Sakwinska O, Kuhn G, Balmelli C, et al. Genetic diversity and ecological success of *Staphylococcus aureus* strains colonizing humans. *Appl Environ Microbiol.* 2009;75:175–183.
207. Rooijakers SH, Ruyken M, Roos A, et al. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. *Nat Immunol.* 2005;6:920–927.
208. Katayama Y, Baba T, Sekine M, et al. Beta-hemolysin promotes skin colonization by *Staphylococcus aureus*. *J Bacteriol.* 2013;195:1194–1203.
209. Verkade E, Bergmans AM, Budding AE, et al. Recent emergence of *Staphylococcus aureus* clonal complex 398 in human blood cultures. *PLoS ONE.* 2012;7:e41855.
210. Larsen J, Petersen A, Sorum M, et al. Methicillin-resistant *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock contact in Denmark, 1999 to 2011. *Euro Surveill.* 2015;20.
211. Brennan GI, Abbott Y, Burns A, et al. The emergence and spread of multiple livestock-associated clonal complex 398 methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains among animals and humans in the Republic of Ireland, 2010–2014. *PLoS ONE.* 2016;11:e0149396.
212. Wardyn SE, Forshey BM, Farina SA, et al. Swine farming is a risk factor for infection with and high prevalence of carriage of multidrug-resistant *Staphylococcus aureus*. *Clin Infect Dis.* 2015;61:59–66.
213. Lowder BV, Guinane CM, Ben Zakour NL, et al. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proc Natl Acad Sci USA.* 2009;106:19545–19550.
214. Boss R, Cosandey A, Luini M, et al. Bovine *Staphylococcus aureus*: subtyping, evolution, and zoonotic transfer. *J Dairy Sci.* 2016;99:515–528.

215. Lindsay JA, Ruzin A, Ross HF, et al. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. *Mol Microbiol*. 1998;29:527–543.
216. Ruzin A, Lindsay J, Novick RP. Molecular genetics of SaPI1—a mobile pathogenicity island in *Staphylococcus aureus*. *Mol Microbiol*. 2001;41:365–377.
217. Tormo-Mas MA, Mir I, Shrestha A, et al. Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands. *Nature*. 2010;465:779–782.
218. Ubeda C, Maiques E, Knecht E, et al. Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol Microbiol*. 2005;60:836–844.
219. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, *Staphylococcus* cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2000;44:1549–1555.
220. Ito T, Okuma K, Ma XX, et al. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist Updat*. 2003;6:41–52.
221. Chambers HF, Hartman BJ, Tomasz A. Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant *Staphylococcus aureus* exposed to nafcillin. *J Clin Invest*. 1985;76:325–331.
222. Zhang HZ, Hackbart CJ, Chansky KM, et al. A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci. *Science*. 2001;291:1962–1965.
223. International Working Group on the Classification of Staphylococcal Cassette Chromosome E. Classification of staphylococcal cassette chromosome *mec* (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother*. 2009;53:4961–4967.
224. Wu Z, Li F, Liu D, et al. Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinant, CcrC2. *Antimicrob Agents Chemother*. 2015;59:7597–7601.
225. Ito T, Katayama Y, Asada K, et al. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2001;45:1323–1336.
226. Ma XX, Ito T, Tiensasitorn C, et al. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother*. 2002;46:1147–1152.
227. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet*. 2006;367:731–731.
228. Diep BA, Stone GG, Basuino L, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis*. 2008;197:1523–1530.
229. Bouchiat C, Curtis S, Spiliopoulou I, et al. MRSA infections among patients in the emergency department: a European multicentre study. *J Antimicrob Chemother*. 2017;72:372–375.
230. Glaser P, Martins-Simoes P, Villain A, et al. Demography and intercontinental spread of the USA300 community-acquired methicillin-resistant *Staphylococcus aureus* lineage. *MBio*. 2016;7:e02183–02115.
231. Petersen A, Stegger M, Heltberg O, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. *Clin Microbiol Infect*. 2013;19:E16–E22.
232. Ba X, Harrison EM, Lovering AL, et al. Old drugs to treat resistant bugs: methicillin-resistant *Staphylococcus aureus* isolates with *mecC* are susceptible to a combination of penicillin and clavulanic acid. *Antimicrob Agents Chemother*. 2015;59:7396–7404.
233. Francioli M, Bille J, Glaeser MP, et al. Beta-lactam resistance mechanisms of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis*. 1991;163:514–523.
234. Mancini S, Laurent F, Veloso TR, et al. In vivo effect of flucloxacillin in experimental endocarditis caused by *mecC*-positive *Staphylococcus aureus* showing temperature-dependent susceptibility in vitro. *Antimicrob Agents Chemother*. 2015;59:2435–2438.
235. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest*. 2003;111:1265–1273.
236. Fowler VG Jr, Boucher HW, Corey GR, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N Engl J Med*. 2006;355:653–665.
237. Abbas M, Paul M, Huttner A. New and improved? A review of novel antibiotics for Gram-positive bacteria. *Clin Microbiol Infect*. 2017;23:697–703.
238. Watanabe S, Ohnishi T, Yuasa A, et al. The first nationwide surveillance of antibacterial susceptibility patterns of pathogens isolated from skin and soft-tissue infections in dermatology departments in Japan. *J Infect Chemother*. 2017;23:503–511.
239. Goffin C, Ghysen JM. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microbiol Mol Biol Rev*. 1998;62:1079–1093.
240. Gillespie MT, May JW, Skurray RA. Antibiotic resistance in *Staphylococcus aureus* isolated at an Australian hospital between 1946 and 1981. *J Med Microbiol*. 1985;19:137–147.
241. Stefanaki C, Ieronymaki A, Matoula T, et al. Six-Year Retrospective Review of Hospital Data on Antimicrobial Resistance Profile of *Staphylococcus aureus* Isolated from Skin Infections from a Single Institution in Greece. *Antibiotics (Basel)*. 2017;6.
242. Richter SS, Doern GV, Heilmann KP, et al. Detection and Prevalence of Penicillin-Susceptible *Staphylococcus aureus* in the United States in 2013. *J Clin Microbiol*. 2016;54:812–814.
243. Nguyen HM, Jones RN. Treatment of methicillin-susceptible *Staphylococcus aureus* osteoarticular and prosthetic joint infections: using the oxacillin minimum inhibitory concentration to guide appropriate ceftriaxone use. *Clin Infect Dis*. 2013;57:161–162.
244. Kang N, Housman ST, Nicolau DP. Assessing the Surrogate Susceptibility of Oxacillin and Cefoxitin for Commonly Utilized Parenteral Agents against Methicillin-Susceptible *Staphylococcus aureus*: focus on Ceftriaxone Discordance between Predictive Susceptibility and in Vivo Exposures. *Pathogens*. 2015;4:599–605.
245. Jeavons MP, Coe AW, Parker MT. Methicillin resistance in staphylococci. *Lancet*. 1963;1:904–907.
246. Panlilio AL, Culver DH, Gaynes RP, et al. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975–1991. *Infect Control Hosp Epidemiol*. 1992;13:582–586.
247. Song Y, Du X, Li T, et al. Phenotypic and molecular characterization of *Staphylococcus aureus* recovered from different clinical specimens of inpatients at a teaching hospital in Shanghai between 2005 and 2010. *J Med Microbiol*. 2013;62(Pt 2):274–282.
248. Klein EY, Mojica N, Jiang W, et al. Trends in methicillin-resistant *Staphylococcus aureus* hospitalizations in the United States, 2010–2014. *Clin Infect Dis*. 2017;65:1921–1923.
249. Walter J, Noll I, Feig M, et al. Decline in the proportion of methicillin resistance among *Staphylococcus aureus* isolates from non-invasive samples and in outpatient settings, and changes in the co-resistance profiles: an analysis of data collected within the Antimicrobial Resistance Surveillance Network, Germany 2010 to 2015. *BMC Infect Dis*. 2017;17:169.
250. Li L, Fortin E, Tremblay C, et al. Hospital-acquired methicillin-resistant *Staphylococcus aureus* bloodstream infections in Quebec: impact of guidelines. *Infect Control Hosp Epidemiol*. 2017;38:840–847.
251. Rolain JM, Abat C, Brouqui P, et al. Worldwide decrease in methicillin-resistant *Staphylococcus aureus*: do we understand something? *Clin Microbiol Infect*. 2015;21:515–517.
252. Gustave CA, Tristan A, Martins-Simoes P, et al. Demographic fluctuation of community-acquired antibiotic-resistant *Staphylococcus aureus* lineages: potential role of flimsy antibiotic exposure. *ISME J*. 2018;8:1879–1894.
253. Johnson AC, Jurgens MD, Nakada N, et al. Linking changes in antibiotic effluent concentrations to flow, removal and consumption in four different UK sewage treatment plants over four years. *Environ Pollut*. 2017;220(Pt B):919–926.
254. DeLeo FR, Otto M, Kreiswirth BN, et al. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet*. 2010;375:1557–1568.
255. Dietrich DW, Auld DB, Mermel LA. Community-acquired methicillin-resistant *Staphylococcus aureus* in southern New England children. *Pediatrics*. 2004;113:e347–e352.
256. Salgado CD, Farr BM, Calfee DP. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin Infect Dis*. 2003;36:131–139.
257. Qiao Y, Dong F, Song W, et al. Hospital- and community-associated methicillin-resistant *Staphylococcus aureus*: a 6-year surveillance study of invasive infections in Chinese children. *Acta Paediatr*. 2013;102:1081–1086.
258. Miller LG, Perdreau-Remington F, Rieg G, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *N Engl J Med*. 2005;352:1445–1453.
259. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9:978–984.
260. Coombs GW, Daly DA, Pearson JC, et al. Community-onset *Staphylococcus aureus* Surveillance Programme annual report, 2012. *Commun Dis Intell Q Rep*. 2014;38:E59–E69.
261. Diekema DJ, Richter SS, Heilmann KP, et al. Continued emergence of USA300 methicillin-resistant *Staphylococcus aureus* in the United States: results from a nationwide surveillance study. *Infect Control Hosp Epidemiol*. 2014;35:285–292.
262. Strauss L, Stegger M, Akpaka PE, et al. Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. *Proc Natl Acad Sci USA*. 2017;114:E10596–E10604.
263. Williamson DA, Roberts SA, Ritchie SR, et al. Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in New Zealand: rapid emergence of sequence type 5 (ST5)-SCCmec-IV as the dominant community-associated MRSA clone. *PLoS ONE*. 2013;8:e62020.
264. Labandeira-Rey M, Couzon F, Boisset S, et al. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. *Science*. 2007;315:1130–1133.
265. Voyich JM, Otto M, Mathema B, et al. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis*. 2006;194:1761–1770.
266. Spaan AN, Henry T, van Rooijen WJ, et al. The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. *Cell Host Microbe*. 2013;13:584–594.
267. Prince A, Wang H, Kitur K, et al. Humanized mice exhibit increased susceptibility to *Staphylococcus aureus* pneumonia. *J Infect Dis*. 2017;215:1386–1395.
268. Rouzi N, Janvier F, Libert N, et al. Prompt and successful toxin-targeting treatment of three patients with necrotizing pneumonia due to *Staphylococcus aureus* strains carrying the Panton-Valentine leukocidin genes. *J Clin Microbiol*. 2010;48:1952–1955.
269. Gauduchon V, Cozon G, Vandenesch F, et al. Neutralization of *Staphylococcus aureus* Panton Valentine leukocidin by intravenous immunoglobulin in vitro. *J Infect Dis*. 2004;189:346–353.
270. Hryniwicz MM, Garbacz K. Borderline oxacillin-resistant *Staphylococcus aureus* (BORA) - a more common problem than expected? *J Med Microbiol*. 2017;66:1367–1373.
271. de Jonge BL, Tomasz A. Abnormal peptidoglycan produced in a methicillin-resistant strain of *Staphylococcus aureus* grown in the presence of methicillin: functional role for penicillin-binding protein 2A in cell wall synthesis. *Antimicrob Agents Chemother*. 1993;37:342–346.
272. de Lencastre H, Tomasz A. Reassessment of the number of auxiliary genes essential for expression of high-level methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1994;38:2590–2598.
273. Berger-Bachi B. Expression of resistance to methicillin. *Trends Microbiol*. 1994;2:389–393.
274. Pinho MG, de Lencastre H, Tomasz A. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proc Natl Acad Sci USA*. 2001;98:10886–10891.
275. Lim D, Strynadka NC. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat Struct Biol*. 2002;9:870–876.
276. Que YA, Entenza JM, Francioli P, et al. The impact of penicillinase on cefamandole treatment and prophylaxis of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *J Infect Dis*. 1998;177:146–154.
277. Tomasz A. Intelligence coup" for drug designers: crystal structure of *Staphylococcus aureus* beta-lactam resistance protein PBP2A. *Lancet*. 2003;361:795–796.
278. Guignard B, Entenza JM, Moreillon P. Beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Curr Opin Pharmacol*. 2005;5:479–489.
279. Bal AM, David MJ, Garav J, et al. Future trends in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection: an in-depth review of newer antibiotics active against an enduring pathogen. *J Glob Antimicrob Resist*. 2017;10:295–303.
280. Chang FY, Peacock JE Jr, Musher DM, et al. *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. *Medicine (Baltimore)*. 2003;82:333–339.

281. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard; 2012.
282. Soriano A, Marco F, Martinez JA, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteraemia. *Clin Infect Dis.* 2008;46:193–200.
283. Mavros MN, Tansarli GS, Vardakas KZ, et al. Impact of vancomycin minimum inhibitory concentration on clinical outcomes of patients with vancomycin-susceptible *Staphylococcus aureus* infections: a meta-analysis and meta-regression. *Int J Antimicrob Agents.* 2012;40:496–509.
284. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health Syst Pharm.* 2009;66:82–98.
285. Song KH, Kim M, Kim CJ, et al. Impact of Vancomycin MIC on Treatment Outcomes in Invasive *Staphylococcus aureus* Infections. *Antimicrob Agents Chemother.* 2017; 61.
286. Hiramatsu K, Hanaki H, Ino T, et al. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother.* 1997;40:135–136.
287. Sieradzki K, Roberts RB, Haber SW, et al. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N Engl J Med.* 1999;340:517–523.
288. Hiramatsu K, Aritaka N, Hanaki H, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneous resistant to vancomycin. *Lancet.* 1997;350:1670–1673.
289. Charles PG, Ward PB, Johnson PD, et al. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate *Staphylococcus aureus*. *Clin Infect Dis.* 2004;38:448–451.
290. Pereira PM, Filipe SR, Tomasaz A, et al. Fluorescence ratio imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in vancomycin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2007;51:3627–3633.
291. Kuroda M, Kuroda H, Oshima T, et al. Two-component system VrsASR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. *Mol Microbiol.* 2003;49:807–821.
292. Neoh HM, Cui L, Yuzawa H, et al. Mutated response regulator graR is responsible for phenotypic conversion of *Staphylococcus aureus* from heterogeneous vancomycin-intermediate resistance to vancomycin-intermediate resistance. *Antimicrob Agents Chemother.* 2008;52:45–53.
293. Howden BP, Stinear TP, Allen DL, et al. Genomic analysis reveals a point mutation in the two-component sensor gene graS that leads to intermediate vancomycin resistance in clinical *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2008;52:3755–3762.
294. Howden BP, McEvoy CR, Allen DL, et al. Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR. *PLoS Pathog.* 2011;7:e1002359.
295. Chen CJ, Huang YC, Chiu CH. Multiple pathways of cross-resistance to glycopeptides and daptomycin in persistent MRSA bacteraemia. *J Antimicrob Chemother.* 2015;70:2965–2972.
296. Dubrac S, Bisicchia P, Devine KM, et al. A matter of life and death: cell wall homeostasis and the WalKR (YycGF) essential signal transduction pathway. *Mol Microbiol.* 2008;70:1307–1322.
297. Moreillon P, Bizzini A, Gidley M, et al. Vancomycin-intermediate *Staphylococcus aureus* selected during vancomycin therapy of experimental endocarditis are not detected by culture-based diagnostic procedures and persist after treatment arrest. *J Antimicrob Chemother.* 2012;67:652–660.
298. Yusof A, Engelhardt A, Karlsson A, et al. Evaluation of a new Etest vancomycin-teicoplanin strip for detection of glycopeptide-intermediate *Staphylococcus aureus* (GISA), in particular, heterogeneous GISA. *J Clin Microbiol.* 2008;46:3042–3047.
299. Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother.* 1993;37:1563–1571.
300. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett.* 1992;72:195–198.
301. Walters MS, Eggers P, Albrecht V, et al. Vancomycin-Resistant *Staphylococcus aureus* - Delaware, 2015. *MMWR Morb Mortal Wkly Rep.* 2015;64:1056.
302. Centers for Disease Control and Prevention. Vancomycin-resistant *Staphylococcus aureus*-New York, 2004. *MMWR Morb Mortal Wkly Rep.* 2004;53:322–323.
303. Dezfulian A, Aslani MM, Oskoui M, et al. Identification and characterization of a high vancomycin-resistant *Staphylococcus aureus* harboring VanA gene cluster isolated from diabetic foot ulcer. *Iran J Basic Med Sci.* 2012;15:803–806.
304. Rossi F, Diaz L, Wollam A, et al. Transferable vancomycin resistance in a community-associated MRSA lineage. *N Engl J Med.* 2014;370:1524–1531.
305. Panesso D, Planet PJ, Diaz L, et al. Methicillin-Susceptible, Vancomycin-Resistant *Staphylococcus aureus*, Brazil. *Emerg Infect Dis.* 2015;21:1844–1848.
306. Iggen B. VanA-type MRSA (VRSA) emerged in surface waters. *Bull Environ Contam Toxicol.* 2016;97:359–366.
307. Scott WR, Baek SB, Jung D, et al. NMR structural studies of the antibiotic lipopeptide daptomycin in DHPC micelles. *Biochim Biophys Acta.* 2007;1768:3116–3126.
308. Baltz RH, Miao W, Wrigley SK. Natural products to drugs: daptomycin and related lipopeptide antibiotics. *Nat Prod Rep.* 2005;22:717–741.
309. Murray KP, Zhao JJ, Davis SL, et al. Early use of daptomycin versus vancomycin for methicillin-resistant *Staphylococcus aureus* bacteremia with vancomycin minimum inhibitory concentration >1 mg/L: a matched cohort study. *Clin Infect Dis.* 2013;56:1562–1569.
310. Carugati M, Bayer AS, Miro JM, et al. High-dose daptomycin therapy for left-sided infective endocarditis: a prospective study from the international collaboration on endocarditis. *Antimicrob Agents Chemother.* 2013;57:6213–6222.
311. Seaton RA, Gonzalez-Ruiz A, Cleveland KO, et al. Real-world daptomycin use across wide geographical regions: results from a pooled analysis of CORE and EU-CORE. *Ann Clin Microbiol Antimicrob.* 2016;15:18.
312. Guleri A, Utili R, Dohmen P, et al. Daptomycin for the treatment of infective endocarditis: results from European Cubicin((R)) Outcomes Registry and Experience (EU-CORE). *Infect Dis Ther.* 2015;4:283–296.
313. Silverman JA, Mortin LI, Vanpraagh AD, et al. Inhibition of daptomycin by pulmonary surfactant: in vitro modeling and clinical impact. *J Infect Dis.* 2005;191:2149–2152.
314. Rose WE, Leonard SN, Sakoulas G, et al. Daptomycin activity against *Staphylococcus aureus* following vancomycin exposure in an in vitro pharmacodynamic model with simulated endocardial vegetations. *Antimicrob Agents Chemother.* 2008;52:831–836.
315. Cui L, Tominaga E, Neoh HM, et al. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2006;50:1079–1082.
316. Patel JB, Jeavitt LA, Hageman J, et al. An association between reduced susceptibility to daptomycin and reduced susceptibility to vancomycin in *Staphylococcus aureus*. *Clin Infect Dis.* 2006;42:1652–1653.
317. Roch M, Gagetti P, Davis J, et al. Daptomycin resistance in clinical MRSA strains is associated with a high biological fitness cost. *Front Microbiol.* 2017;8:2303.
318. Renzoni A, Kelley WL, Rosato RR, et al. Molecular bases determining daptomycin resistance-mediated resensitization to beta-lactams (seesaw effect) in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2017;61.
319. Shafiq I, Bulman ZP, Spitznogle SL, et al. A combination of ceftazidime and daptomycin has synergistic and bactericidal activity in vitro against daptomycin nonsusceptible methicillin-resistant *Staphylococcus aureus* (MRSA). *Infect Dis (Lond).* 2017;49:410–416.
320. Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother.* 1995;39:577–585.
321. Levin TP, Suh B, Axelrod P, et al. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant *Staphylococcus aureus*: report of a clinical failure. *Antimicrob Agents Chemother.* 2005;49:1222–1224.
322. Putnam SD, Sader HS, Farrell DJ, et al. Antimicrobial characterisation of solithromycin (CEM-101), a novel fluoroketolide: activity against staphylococci and enterococci. *Int J Antimicrob Agents.* 2011;37:39–45.
323. Davis KA, Crawford SA, Fiebelkorn KR, et al. Induction of telithromycin resistance by erythromycin in isolates of macrolide-resistant *Staphylococcus* spp. *Antimicrob Agents Chemother.* 2005;49:3059–3061.
324. Schaumburg F, Idelevich EA, Peters G, et al. Trends in antimicrobial non-susceptibility in methicillin-resistant *Staphylococcus aureus* from Germany (2004–2011). *Clin Microbiol Infect.* 2014;20:O554–O557.
325. Ross JI, Eady EA, Cove JH, et al. Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter Msra may interact. *Gene.* 1995;153:93–98.
326. Clancy J, Pettipas J, Dib-Hajj F, et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes*. *Mol Microbiol.* 1996;22:867–879.
327. Vallianou N, Evangelopoulos A, Hadjisoteriou M, et al. Prevalence of macrolide, lincosamide, and streptogramin resistance among staphylococci in a tertiary care hospital in Athens, Greece. *J Chemother.* 2015;27:319–323.
328. Shashindran N, Nagasundaram N, Thappa DM, et al. Can Panton Valentine leukocidin gene and clindamycin susceptibility serve as predictors of community origin of MRSA from skin and soft tissue infections? *J Clin Diagn Res.* 2016;10:DC1–DC4.
329. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis.* 2012;54:621–629.
330. Falagas ME, Manta KG, Ntziora F, et al. Linezolid for the treatment of patients with endocarditis: a systematic review of the published evidence. *J Antimicrob Chemother.* 2006;58:273–280.
331. Watkins RR, Lemonovich TL, File TM Jr. An evidence-based review of linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): place in therapy. *Core Evid.* 2012;7:131–143.
332. Chen CJ, Chiu CH, Lin TY, et al. Experience with linezolid therapy in children with osteoarticular infections. *Pediatr Infect Dis J.* 2007;26:985–988.
333. Stevens DL, Ma Y, Salmi DB, et al. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *J Infect Dis.* 2007;195:202–211.
334. Beekmann SE, Gilbert DN, Polgreen PM, et al. Toxicity of extended courses of linezolid: results of an Infectious Diseases Society of America Emerging Infections Network survey. *Diagn Microbiol Infect Dis.* 2008;62:407–410.
335. Meka VG, Gold HS. Antimicrobial resistance to linezolid. *Clin Infect Dis.* 2004;39:1010–1015.
336. Mendes RE, Deshpande LM, Castanheira M, et al. First report of cfr-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob Agents Chemother.* 2008;52:2244–2246.
337. Long KS, Poehlsgaard J, Kehrenberg C, et al. The Cfr rRNA methyltransferase confers resistance to Phenicons, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrob Agents Chemother.* 2006;50:2500–2505.
338. Mendes RE, Deshpande LM, Bonilla HF, et al. Dissemination of a pSCFS3-like cfr-carrying plasmid in *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical isolates recovered from hospitals in Ohio. *Antimicrob Agents Chemother.* 2013;57:2923–2928.
339. Shore AC, Lazaris A, Kinnevey PM, et al. First report of cfr-carrying plasmids in the pandemic sequence type 22 methicillin-resistant *Staphylococcus aureus* staphylococcal cassette chromosome mec type IV clone. *Antimicrob Agents Chemother.* 2016;60:3007–3015.
340. Flamm RK, Mendes RE, Hogan PA, et al. Linezolid surveillance results for the United States (LEADER surveillance program 2014). *Antimicrob Agents Chemother.* 2016;60:2273–2280.
341. Sader HS, Mendes RE, Streit JM, et al. Antimicrobial susceptibility trends among *Staphylococcus aureus* isolates from U.S. hospitals: results from 7 years of the ceftazidime (AWARE) surveillance program, 2010 to 2016. *Antimicrob Agents Chemother.* 2017;61.
342. Rybak JM, Roberts K. Tedizolid phosphate: a next-generation oxazolidinone. *Infect Dis Ther.* 2015.
343. Lepak AJ, Marchillo K, Pichereau S, et al. Comparative pharmacodynamics of the new oxazolidinone tedizolid phosphate and linezolid in a neutropenic murine *Staphylococcus aureus* pneumonia model. *Antimicrob Agents Chemother.* 2012;56:5916–5922.
344. Shorr AF, Lodise TP, Corey GR, et al. Analysis of the phase 3 ESTABLISH trials of tedizolid versus linezolid in acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother.* 2015;59:864–871.
345. Dholakia N, Rolston KV, Ho DH, et al. Susceptibilities of bacterial isolates from patients with cancer to levofloxacin and other quinolones. *Antimicrob Agents Chemother.* 1994;38:848–852.
346. Coombs GW, Nimmo GR, Daly DA, et al. Australian *Staphylococcus aureus* Sepsis Outcome Programme annual report, 2013. *Commun Dis Intell Q Rep.* 2014;38:E309–E319.
347. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis.* 2005;41 Suppl 2:S120–S126.

348. Arsene S, Leclercq R. Role of a qnr-like gene in the intrinsic resistance of *Enterococcus faecalis* to fluoroquinolones. *Antimicrob Agents Chemother*. 2007;51:3254–3258.
349. Yoshida H, Bogaki M, Nakamura S, et al. Nucleotide sequence and characterization of the *Staphylococcus aureus* norA gene, which confers resistance to quinolones. *J Bacteriol*. 1990;172:6942–6949.
350. Fournier B, Hooper DC. Mutations in topoisomerase IV and DNA gyrase of *Staphylococcus aureus*: novel pleiotropic effects on quinolone and coumarin activity. *Antimicrob Agents Chemother*. 1998;42:121–128.
351. Entenza JM, Vouillamoz J, Glaser MP, et al. Levofloxacin versus ciprofloxacin, flucloxacillin, or vancomycin for treatment of experimental endocarditis due to methicillin-susceptible or -resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1997;41:1662–1667.
352. Remy JM, Tow-Keogh CA, McConnell TS, et al. Activity of delafloxacin against methicillin-resistant *Staphylococcus aureus*: resistance selection and characterization. *J Antimicrob Chemother*. 2012;67:2814–2820.
353. Craig WA. Does the dose matter? *Clin Infect Dis*. 2001;33 Suppl 3:S233–S237.
354. Cui J, Liu Y, Wang R, et al. The mutant selection window in rabbits infected with *Staphylococcus aureus*. *J Infect Dis*. 2006;194:1601–1608.
355. Chopra I. Glycylcyclines: third-generation tetracycline antibiotics. *Curr Opin Pharmacol*. 2001;1:464–469.
356. Yang Q, Xu YC, Kiratisin P, et al. Antimicrobial activity among gram-positive and gram-negative organisms collected from the Asia-Pacific region as part of the Tigecycline Evaluation and Surveillance Trial: comparison of 2015 results with previous years. *Diagn Microbiol Infect Dis*. 2017;89:314–323.
357. Yahav D, Lador A, Paul M, et al. Efficacy and safety of tigecycline: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2011;66:1963–1971.
358. Cocio C, Di Giambattista M, Nyssen E, et al. Inhibition of protein synthesis by streptogramins and related antibiotics. *J Antimicrob Chemother*. 1997;39 Suppl A:7–13.
359. Vouillamoz J, Entenza JM, Feger C, et al. Quinupristin-dalfopristin combined with beta-lactams for treatment of experimental endocarditis due to *Staphylococcus aureus* constitutively resistant to macrolide-lincosamide-streptogramin B antibiotics. *Antimicrob Agents Chemother*. 2000;44:1789–1795.
360. Billeter M, Zervos MJ, Chen AY, et al. Kurukularatne C, Dalbavancin: a novel once-weekly lipoglycopeptide antibiotic. *Clin Infect Dis*. 2008;46:577–583.
361. Allen NE, Nicolas TL. Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microbiol Rev*. 2003;26:511–532.
362. Al Jalali V, Zeitzlinger M. Clinical Pharmacokinetics and Pharmacodynamics of Telavancin Compared with the Other Glycopeptides. *Clin Pharmacokinet*. 2018.
363. Jauregui LE, Babazadeh S, Seltzer E, et al. Randomized, double-blind comparison of once-weekly dalbavancin versus twice-daily linezolid therapy for the treatment of complicated skin and skin structure infections. *Clin Infect Dis*. 2005;41:1407–1415.
364. Corey GR, Good S, Jiang H, et al. Single-dose oritavancin versus 7–10 days of vancomycin in the treatment of gram-positive acute bacterial skin and skin structure infections: the SOLO II noninferiority study. *Clin Infect Dis*. 2015;60:254–262.
365. Cremieux AC, Maziere B, Vallois JM, et al. Evaluation of antibiotic diffusion into cardiac vegetations by quantitative autoradiography. *J Infect Dis*. 1989;159:938–944.
366. Steele JM, Seabury RW, Hale CM, et al. Unsuccessful treatment of methicillin-resistant *Staphylococcus aureus* endocarditis with dalbavancin. *J Clin Pharm Ther*. 2018;43:101–103.
367. Falagas ME, Matthaiou DK, Bliziotis IA. The role of aminoglycosides in combination with a beta-lactam for the treatment of bacterial endocarditis: a meta-analysis of comparative trials. *J Antimicrob Chemother*. 2006;57:639–647.
368. Davis JS, Van Hal S, Tong SY. Combination antibiotic treatment of serious methicillin-resistant *Staphylococcus aureus* infections. *Semin Respir Crit Care Med*. 2015;36:3–16.
369. Davis JS, Sud A, O'Sullivan MVN, et al. Combination of vancomycin and beta-lactam therapy for methicillin-resistant *Staphylococcus aureus* bacteremia: a pilot multicenter randomized controlled trial. *Clin Infect Dis*. 2016;62:173–180.
370. Tong SY, Nelson J, Paterson DL, et al. CAMERA2 – combination antibiotic therapy for methicillin-resistant *Staphylococcus aureus* infection: study protocol for a randomised controlled trial. *Trials*. 2016;17:170.
371. Pericas JM, Moreno A, Almela M, et al. Efficacy and safety of fosfomycin plus imipenem versus vancomycin for complicated bacteraemia and endocarditis due to methicillin-resistant *Staphylococcus aureus*: a randomized clinical trial. *Clin Microbiol Infect*. 2018.
372. Fuda C, Hesek D, Lee M, et al. Mechanistic basis for the action of new cephalosporin antibiotics effective against methicillin- and vancomycin-resistant *Staphylococcus aureus*. *J Biol Chem*. 2006;281:10035–10041.
373. Corey GR, Wilcox MH, Talbot GH, et al. CANVAS 1: the first Phase III, randomized, double-blind study evaluating ceftaroline fosamil for the treatment of patients with complicated skin and skin structure infections. *J Antimicrob Chemother*. 2010;65 Suppl 4:iv41–iv51.
374. Vidaillac C, Leonard SN, Rybak MJ. In vitro activity of ceftaroline against methicillin-resistant *Staphylococcus aureus* and heterogeneous vancomycin-intermediate *S. aureus* in a hollow fiber model. *Antimicrob Agents Chemother*. 2009;53:4712–4717.
375. Sakoulas G, Moise PA, Casapao AM, et al. Antimicrobial salvage therapy for persistent staphylococcal bacteraemia using daptomycin plus ceftaroline. *Clin Ther*. 2014;36:1317–1333.
376. Valour F, Trouillet-Assant S, Riffard N, et al. Antimicrobial activity against intraosteoblastic *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2015;59:2029–2036.
377. Ho TT, Cadena J, Childs LM, et al. Methicillin-resistant *Staphylococcus aureus* bacteraemia and endocarditis treated with ceftaroline salvage therapy. *J Antimicrob Chemother*. 2012;67:1267–1270.
378. Pasquale TR, Tan MJ, Trienski TL, et al. Methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia patients treated with ceftaroline: retrospective case series of 10 patients. *J Chemother*. 2015;27:29–34.
379. Pagani L, Bonnin P, Janssen C, et al. Ceftaroline for the treatment of prosthetic valve endocarditis due to methicillin-resistant *Staphylococcus aureus*. *J Heart Valve Dis*. 2014;23:219–221.
380. Lin JC, Aung G, Thomas A, et al. The use of ceftaroline fosamil in methicillin-resistant *Staphylococcus aureus* endocarditis and deep-seated MRSA infections: a retrospective case series of 10 patients. *J Infect Chemother*. 2013;19:42–49.
381. Perumal Samy R, Stiles BG, Franco OL, et al. Animal venoms as antimicrobial agents. *Biochem Pharmacol*. 2017;134:127–138.
382. de Breij A, Riolo M, Cordfunke RA, et al. The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms. *Sci Transl Med*. 2018;10.
383. Tajbakhsh M, Karimi A, Tohidpour A, et al. The antimicrobial potential of a new derivative of cathelicidin from *Bungarus fasciatus* against methicillin-resistant *Staphylococcus aureus*. *J Microbiol*. 2018;56:128–137.
384. Torres PJ, Pishchany G, Humayun M, et al. *Staphylococcus aureus* IsdB is a hemoglobin receptor required for heme iron utilization. *J Bacteriol*. 2006;188:8421–8429.
385. McAdow M, Kim HK, Dedent AC, et al. Preventing *Staphylococcus aureus* sepsis through the inhibition of its agglutination in blood. *PLoS Pathog*. 2011;7:e1002307.
386. Sully EK, Malachowa N, Elmore BO, et al. Selective chemical inhibition of agr quorum sensing in *Staphylococcus aureus* promotes host defense with minimal impact on resistance. *PLoS Pathog*. 2014;10:e1004174.
387. Raz A, Serrano A, Lawson C, et al. Lysibodies are IgG Fc fusions with lysin binding domains targeting *Staphylococcus aureus* wall carbohydrates for effective phagocytosis. *Proc Natl Acad Sci USA*. 2017;114:4781–4786.
388. Ko HHT, Lareu RR, Dix BR, et al. Statins: antimicrobial resistance breakers or makers? *PeerJ*. 2017;5:e3952.
389. Janda S, Young A, Fitzgerald JM, et al. The effect of statins on mortality from severe infections and sepsis: a systematic review and meta-analysis. *J Crit Care*. 2010;25:656, e7–e22.
390. Caffrey AR, Timbrook TT, Noh E, et al. Evidence To Support Continuation of Statin Therapy in Patients with *Staphylococcus aureus* Bacteremia. *Antimicrob Agents Chemother*. 2017;61.
391. Garcia-Fernandez E, Koch G, Wagner RM, et al. Membrane microdomain disassembly inhibits MRSA antibiotic resistance. *Cell*. 2017;171:1354–1367, e1320.
392. Thangamani S, Mohammad H, Abushahba MF, et al. Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. *Sci Rep*. 2015;5:16407.
393. Lehar SM, Pillow T, Xu M, et al. Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. *Nature*. 2015;527:323–328.
394. Fischetti VA. Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens. *Int J Med Microbiol*. 2010;300:357–362.
395. Nobrega FL, Costa AR, Kluskens LD, et al. Revisiting phage therapy: new applications for old resources. *Trends Microbiol*. 2015;23:185–191.
396. Pastagia M, Euler C, Chahales P, et al. A novel chimeric lytic shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus* strains. *Antimicrob Agents Chemother*. 2011;55:738–744.
397. Yang H, Zhang Y, Yu J, et al. Novel chimeric lytic with high-level antimicrobial activity against methicillin-resistant *Staphylococcus aureus* in vitro and in vivo. *Antimicrob Agents Chemother*. 2014;58:536–542.
398. Channabasappa S, Durgaiah M, Chikkamadaiah R, et al. Efficacy of novel antistaphylococcal ectolysin P128 in a rat model of methicillin-resistant *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother*. 2018;62.
399. Totte JEE, van Doorn MB, Pasmans S. Successful treatment of chronic *Staphylococcus aureus*-related dermatoses with the topical endolysin staphefekt SA. 100: a report of 3 cases. *Case Rep Dermatol*. 2017;9:19–25.
400. Totte J, de Wit J, Pardo L, et al. Targeted anti-staphylococcal therapy with endolysins in atopic dermatitis and the effect on steroid use, disease severity and the microbiome: study protocol for a randomized controlled trial (MAAS trial). *Trials*. 2017;18:404.
401. Cisek AA, Dabrowska I, Gregorczyk KP, et al. Phage therapy in bacterial infections treatment: one hundred years after the discovery of bacteriophages. *Curr Microbiol*. 2017;74:277–283.
402. Oechslin F, Piccardi P, Mancini S, et al. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J Infect Dis*. 2017;215:703–712.
403. Fish R, Kutter E, Wheat G, et al. Bacteriophage treatment of intransigent diabetic toe ulcers: a case series. *J Wound Care*. 2016;25 Suppl 7:S27–S33.
404. Shinefield H, Black S, Fattom A, et al. Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *N Engl J Med*. 2002;346:491–496.
405. Fowler VG, Allen KB, Moreira ED, et al. Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. *JAMA*. 2013;309:1368–1378.
406. Fitzgerald JR, Loughman A, Keane F, et al. Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the Fc gamma RIa receptor. *Mol Microbiol*. 2006;59:212–230.
407. Spaulding AR, Salgado-Pabon W, Merriman JA, et al. Vaccination against *Staphylococcus aureus* pneumonia. *J Infect Dis*. 2014;209:1955–1962.
408. Mohamed N, Wang MY, Le Huec JC, et al. Vaccine development to prevent *Staphylococcus aureus* surgical-site infections. *Br J Surg*. 2017;104:e41–e54.
409. Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *Infect Control Hosp Epidemiol*. 2016;37:1288–1301.
410. Magill SS, Edwards JR, Bamberg W, et al. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. 2014;370:1198–1208.
411. Dantes R, Mu Y, Belflower R, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Intern Med*. 2013;173:1970–1978.
412. Malani PN. National burden of invasive methicillin-resistant *Staphylococcus aureus* infection. *JAMA*. 2014;311:1438–1439.
413. Laupland KB, Church DL, Mucenski M, et al. Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. *J Infect Dis*. 2003;187:1452–1459.
414. van Hal SJ, Jensen SO, Vasku VL, et al. Predictors of mortality in *Staphylococcus aureus* Bacteremia. *Clin Microbiol Rev*. 2012;25:362–386.
415. Gott M, Schweizer ML, Vaughan-Sarrazin MS, et al. Association of evidence-based care processes with mortality in *Staphylococcus aureus* bacteremia at Veterans Health Administration hospitals, 2003–2014. *JAMA Intern Med*. 2017;177:1489–1497.
416. Lambert ML, Sueters C, Savy A, et al. Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. *Lancet Infect Dis*. 2011;11:30–38.

417. Nguyen DB, Shugart A, Lines C, et al. National Healthcare Safety Network (NHSN) dialysis event surveillance report for 2014. *Clin J Am Soc Nephrol*. 2017;12:1139–1146.
418. Noskin GA, Rubin RJ, Schentag JJ, et al. National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6-year period (1998–2003). *Clin Infect Dis*. 2007;45:1132–1140.
419. Gidengil CA, Gay C, Huang SS, et al. Cost-effectiveness of strategies to prevent methicillin-resistant *Staphylococcus aureus* transmission and infection in an intensive care unit. *Infect Control Hosp Epidemiol*. 2015;36:17–27.
420. Jacobsson G, Dashti S, Wahlberg T, et al. The epidemiology of and risk factors for invasive *Staphylococcus aureus* infections in western Sweden. *Scand J Infect Dis*. 2007;39:6–13.
421. Diekema DJ, Pfaller MA, Schmitz FJ, et al. 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*. 2001;32 Suppl 2:S114–S132.
422. Fowler VG, Miro JM, Hoen B, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA*. 2005;293:3012–3021.
423. Worth LJ, Bull AL, Spelman T, et al. Diminishing surgical site infections in Australia: time trends in infection rates, pathogens and antimicrobial resistance using a comprehensive Victorian surveillance program, 2002–2013. *Infect Control Hosp Epidemiol*. 2015;36:409–416.
424. Mimoz O, Lucet JC, Kerforne T, et al. Skin antisepsis with chlorhexidine-alcohol versus povidone iodine-alcohol, with and without skin scrubbing, for prevention of intravascular-catheter-related infection (CLEAN): an open-label, multicentre, randomised, controlled, two-by-two factorial trial. *Lancet*. 2015;386:2069–2077.
425. Lee MS, Walker V, Chen LF, et al. The epidemiology of ventilator-associated pneumonia in a network of community hospitals: a prospective multicenter study. *Infect Control Hosp Epidemiol*. 2013;34:657–662.
426. Que YA, Moreillon P. Infective endocarditis. *Nat Rev Cardiol*. 2011;8:322–336.
427. Selton-Suty C, Celard M, Le Moing V, et al. Preeminence of *Staphylococcus aureus* in infective endocarditis: a 1-year population-based survey. *Clin Infect Dis*. 2012;54:1230–1239.
428. Hoen B, Duval X. Clinical practice. Infective endocarditis. *N Engl J Med*. 2013;368:1425–1433.
429. Mathews CJ, Weston VC, Jones A, et al. Bacterial septic arthritis in adults. *Lancet*. 2010;375:846–855.
430. Bernard L, Dinh A, Ghout I, et al. Antibiotic treatment for 6 weeks versus 12 weeks in patients with pyogenic vertebral osteomyelitis: an open-label, non-inferiority, randomised, controlled trial. *Lancet*. 2015;385:875–882.
431. Tanda AJ, Patel R. Prosthetic joint infection. *Clin Microbiol Rev*. 2014;27:302–345.
432. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. *Clin Microbiol Infect*. 2013;19:492–500.
433. Laupland KB, Ross T, Gregson DB. *Staphylococcus aureus* bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000–2006. *J Infect Dis*. 2008;198:336–343.
434. van Rijen M, Bonten M, Wenzel R, et al. Mupirocin ointment for preventing *Staphylococcus aureus* infections in nasal carriers. *Cochrane Database Syst Rev*. 2008;(4):CD006216.
435. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol*. 2008;29:996–1011.
436. Acree ME, Morgan E, David MZ. *S. aureus* infections in Chicago, 2006–2014: increase in CA MSSA and decrease in MRSA incidence. *Infect Control Hosp Epidemiol*. 2017;38:1226–1234.
437. Kallen AJ, Mu Y, Bulsens S, et al. Health care-associated invasive MRSA infections, 2005–2008. *JAMA*. 2010;304:641–648.
438. Landrum ML, Neumann C, Cook C, et al. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005–2010. *JAMA*. 2012;308:50–59.
439. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol*. 2009;7:629–641.
440. Uhlemann AC, Otto M, Lowy FD, et al. Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol*. 2014;21:563–574.
441. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin Infect Dis*. 2006;42 Suppl 2:S82–S89.
442. Yaw LK, Robinson JO, Ho KM. A comparison of long-term outcomes after methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* bacteraemia: an observational cohort study. *Lancet Infect Dis*. 2014;14:967–975.
443. Melles DC, Gorkink RE, Boelens HA, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. *J Clin Invest*. 2004;114:1732–1740.
444. Cameron DR, Howden BP, Peleg AY. The interface between antibiotic resistance and virulence in *Staphylococcus aureus* and its impact upon clinical outcomes. *Clin Infect Dis*. 2011;53:576–582.
445. Miller LG, Daum RS, Creech CB, et al. Clindamycin versus trimethoprim-sulfamethoxazole for uncomplicated skin infections. *N Engl J Med*. 2015;372:1093–1103.
446. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23:616–687.
447. Seybold U, Kourbatova EV, Johnson JG, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health-care-associated blood stream infections. *Clin Infect Dis*. 2006;42:647–656.
448. Jenkins TC, McCollister BD, Sharma R, et al. Epidemiology of healthcare-associated bloodstream infection caused by USA300 strains of methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. *Infect Control Hosp Epidemiol*. 2009;30:233–241.
449. Planet PJ. Life after USA300: the rise and fall of a superbug. *J Infect Dis*. 2017;215(suppl\_1):S71–S77.
450. Mertz D, Frei R, Periat N, et al. Exclusive *Staphylococcus aureus* throat carriage: at-risk populations. *Arch Intern Med*. 2009;169:172–178.
451. van Belkum A, Verkaik NJ, de Vogel CP, et al. Reclassification of *Staphylococcus aureus* nasal carriage types. *J Infect Dis*. 2009;199:1820–1826.
452. Sollid JU, Furberg AS, Hanssen AM, et al. *Staphylococcus aureus*: determinants of human carriage. *Infect Genet Evol*. 2014;21:531–541.
453. Burian M, Wolz C, Goerke C. Regulatory adaptation of *Staphylococcus aureus* during nasal colonization of humans. *PLoS ONE*. 2010;5:e10040.
454. Wertheim HF, Walsh E, Choudhury R, et al. Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. *PLoS Med*. 2008;5:e17.
455. Clarke SR, Mohamed R, Bian L, et al. The *Staphylococcus aureus* surface protein *lslA* mediates resistance to innate defenses of human skin. *Cell Host Microbe*. 2007;1:199–212.
456. Lebon A, Labout JA, Verbrugh HA, et al. Dynamics and determinants of *Staphylococcus aureus* carriage in infancy: The generation R study. *J Clin Microbiol*. 2008.
457. van Gils EJ, Hak E, Veenhoven RH, et al. Effect of seven-valent pneumococcal conjugate vaccine on *Staphylococcus aureus* colonisation in a randomised controlled trial. *PLoS ONE*. 2011;6:e20229.
458. Price JR, Cole K, Bexley A, et al. Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect Dis*. 2017;17:207–214.
459. Simor AE. Staphylococcal decolonisation: an effective strategy for prevention of infection? *Lancet Infect Dis*. 2011;11:952–962.
460. Gorwitz R, Kruszon-Moran D, McAllister S, et al. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. *J Infect Dis*. 2008;197:1226–1234.
461. Fuller C, Robotham J, Savage J, et al. The national one week prevalence audit of universal methicillin-resistant *Staphylococcus aureus* (MRSA) admission screening 2012. *PLoS ONE*. 2013;8:e74219.
462. McKinnell JA, Miller LG, Eells SJ, et al. A systematic literature review and meta-analysis of factors associated with methicillin-resistant *Staphylococcus aureus* colonization at time of hospital or intensive care unit admission. *Infect Control Hosp Epidemiol*. 2013;34:1077–1086.
463. Davis MF, Iverson SA, Baron P, et al. Household transmission of methicillin-resistant *Staphylococcus aureus* and other staphylococci. *Lancet Infect Dis*. 2012;12:703–716.
464. Fatkenheuer G, Hirschel B, Harbarth S. Screening and isolation to control methicillin-resistant *Staphylococcus aureus*: sense, nonsense, and evidence. *Lancet*. 2015;385:1146–1149.
465. Kock R, Becker K, Cookson B, et al. Systematic literature analysis and review of targeted preventive measures to limit healthcare-associated infections by methicillin-resistant *Staphylococcus aureus*. *Euro Surveill*. 2014;19.
466. Robotham JV, Deeny SR, Fuller C, et al. Cost-effectiveness of national mandatory screening of all admissions to English National Health Service hospitals for methicillin-resistant *Staphylococcus aureus*: a mathematical modelling study. *Lancet Infect Dis*. 2016;16:348–356.
467. Loeb M, Main C, Walker-Dilks C, et al. Antimicrobial drugs for treating methicillin-resistant *Staphylococcus aureus* colonization. *Cochrane Database Syst Rev*. 2003;(4):CD003340.
468. Ammerlaan HS, Kluytmans JA, Berkhouit H, et al. Eradication of carriage with methicillin-resistant *Staphylococcus aureus*: effectiveness of a national guideline. *J Antimicrob Chemother*. 2011;66:2409–2417.
469. Albrich WC, Harbarth S. Health-care workers: source, vector, or victim of MRSA? *Lancet Infect Dis*. 2008;8:289–301.
470. Climo MW, Yokoe DS, Warren DK, et al. Effect of daily chlorhexidine bathing on hospital-acquired infection. *N Engl J Med*. 2013;368:533–542.
471. Timits JF, Mimoz O, Mourvillier B, et al. Randomized controlled trial of chlorhexidine dressing and highly adhesive dressing for preventing catheter-related infections in critically ill adults. *Am J Respir Crit Care Med*. 2012;186:1272–1278.
472. Timits JF, Schwobel C, Bouadma L, et al. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA*. 2009;301:1231–1241.
473. Derde LPG, Cooper BS, Goossens H, et al. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: an interrupted time series study and cluster randomised trial. *Lancet Infect Dis*. 2014;14:31–39.
474. Huang SS, Septimus E, Kleinman K, et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med*. 2013;368:2255–2265.
475. Bowen AC, Tong SY, Chatfield MD, et al. The microbiology of impetigo in indigenous children: associations between *Streptococcus pyogenes*, *Staphylococcus aureus*, scabies, and nasal carriage. *BMC Infect Dis*. 2014;14:727.
476. Lee IW, Kang L, Hsu HP, et al. Puerperal mastitis requiring hospitalization during a nine-year period. *Am J Obstet Gynecol*. 2010;203:332, e331–e336.
477. Fernandez L, Cardenes N, Arroyo R, et al. Prevention of infectious mastitis by oral administration of *Lactobacillus salivarius* PS2 during late pregnancy. *Clin Infect Dis*. 2016;62:568–573.
478. Arroyo R, Martin V, Maldonado A, et al. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of *Lactobacilli* isolated from breast milk. *Clin Infect Dis*. 2010;50:1551–1558.
479. Anderson DJ, Podgorny K, Berrios-Torres SI, et al. Strategies to prevent surgical site infections in acute care hospitals: 2014 update. *Infect Control Hosp Epidemiol*. 2014;35:605–627.
480. Allegranzi B, Bischoff P, de Jonge S, et al. New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. *Lancet Infect Dis*. 2016;16:e276–e287.
481. McClain SL, Bohan JG, Stevens DL. Advances in the medical management of skin and soft tissue infections. *BMJ*. 2016;355:i6004.
482. Eggimann P, Sax H, Pittet D. Catheter-related infections. *Microbes Infect*. 2004;6:1033–1042.
483. 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*. 1975;132:316–335.
484. Rodriguez-Creixems M, Alcalá L, Muñoz P, et al. Bloodstream infections: evolution and trends in the microbiology workload, incidence, and etiology, 1985–2006. *Medicine (Baltimore)*. 2008;87:234–249.
485. Laupland KB, Lyytikainen O, Sogaard M, et al. The changing epidemiology of *Staphylococcus aureus* bloodstream infection: a multinational population-based surveillance study. *Clin Microbiol Infect*. 2013;19:465–471.
486. Tong SY, Davis JS, Eichenberger E, et al. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28:603–661.
487. Moreillon P, Que YA. Infective endocarditis. *Lancet*. 2004;363:139–149.
488. Gouello JP, Asfar P, Brenet O, et al. Nosocomial endocarditis in the intensive care unit: an analysis of 22 cases. *Crit Care Med*. 2000;28:377–382.

489. Habib G, Lancellotti P, Antunes MJ, et al. 2015 ESC Guidelines for the management of infective endocarditis: the Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (ECTS), the European Association of Nuclear Medicine (EANM). *Eur Heart J.* 2015;36:3075–3128.
490. Holland TL, Arnold C, Fowler VG Jr. Clinical management of *Staphylococcus aureus* bacteremia: a review. *JAMA.* 2014;312:1330–1341.
491. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. *Circulation.* 2015;132:1435–1486.
492. Fowler VG Jr, Olsen MK, Corey GR, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Intern Med.* 2003;163:2066–2072.
493. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2009;49:1–45.
494. Cosgrove SE, Fowler VG Jr. Management of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis.* 2008;46 Suppl 5:S386–S393.
495. Daneman N, Shore K, Pinto R, et al. Antibiotic treatment duration for bloodstream infections in critically ill patients: a national survey of Canadian infectious diseases and critical care specialists. *Int J Antimicrob Agents.* 2011;38:480–485.
496. Duval X, Delahaye F, Alla F, et al. Temporal trends in infective endocarditis in the context of prophylaxis guideline modifications: three successive population-based surveys. *J Am Coll Cardiol.* 2012;59:1968–1976.
497. Wang A, Athan E, Pappas PA, et al. Contemporary clinical profile and outcome of prosthetic valve endocarditis. *JAMA.* 2007;297:1354–1361.
498. Kamalakannan D, Pai RM, Johnson LB, et al. Epidemiology and clinical outcomes of infective endocarditis in hemodialysis patients. *Ann Thorac Surg.* 2007;83:2081–2086.
499. Benito N, Miro JM, de Lazzari E, et al. Health care-associated native valve endocarditis: importance of non-nosocomial acquisition. *Ann Intern Med.* 2009;150:586–594.
500. Murdoch DR, Corey GR, Hoen B, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. *Arch Intern Med.* 2009;169:463–473.
501. Moreillon P, Que YA, Bayer AS. Pathogenesis of streptococcal and staphylococcal endocarditis. *Infect Dis Clin North Am.* 2002;16:297–318.
502. Que YA, Haefliger JA, Francioli P, et al. Expression of *Staphylococcus aureus* clumping factor A in *Lactococcus lactis* subsp. *cremoris* using a new shuttle vector. *Infect Immun.* 2000;68:3516–3522.
503. Que YA, Francois P, Haefliger JA, et al. Reassessing the role of *Staphylococcus aureus* clumping factor and fibronectin-binding protein by expression in *Lactococcus lactis*. *Infect Immun.* 2001;69:6296–6302.
504. Foster TJ, Geoghegan JA, Ganesh VK, et al. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat Rev Microbiol.* 2014;12:49–62.
505. Josse J, Laurent F, Diot A. Staphylococcal adhesion and host cell invasion: fibronectin-binding and other mechanisms. *Front Microbiol.* 2017;8:2433.
506. Yeaman MR. Platelets at the nexus of antimicrobial defence. *Nat Rev Microbiol.* 2014;12:426–437.
507. Andersson DI, Hughes D, Kubicek-Sutherland JZ. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. *Drug Resist Updat.* 2016;26:43–57.
508. Fowler VG, McIntyre LM, Yeaman MR, et al. In vitro resistance to thrombin-induced platelet microbicidal protein in isolates of *Staphylococcus aureus* from endocarditis patients correlates with an intravascular device source. *J Infect Dis.* 2000;182:1251–1254.
509. Anaya-Lopez JL, Lopez-Meza JE, Ochoa-Zarzosa A. Bacterial resistance to cationic antimicrobial peptides. *Crit Rev Microbiol.* 2013;39:180–195.
510. Veloso TR, Chaouch A, Roger T, et al. Use of a human-like low-grade bacteremia model of experimental endocarditis to study the role of *Staphylococcus aureus* adhesins and platelet aggregation in early endocarditis. *Infect Immun.* 2013;81:697–703.
511. Habib A, Irfan M, Baddour LM, et al. Impact of prior aspirin therapy on clinical manifestations of cardiovascular implantable electronic device infections. *Europace.* 2013;15:227–235.
512. Chan KL, Tam J, Dumesnil JG, et al. Effect of long-term aspirin use on embolic events in infective endocarditis. *Clin Infect Dis.* 2008;46:37–41.
513. Snygg-Martin U, Rasmussen RV, Hassager C, et al. The relationship between cerebrovascular complications and previously established use of antiplatelet therapy in left-sided infective endocarditis. *Scand J Infect Dis.* 2011;43:899–904.
514. Kupferwasser LI, Yeaman MR, Shapiro SM, et al. Acetylsalicylic acid reduces vegetation bacterial density, hematogenous bacterial dissemination, and frequency of embolic events in experimental *Staphylococcus aureus* endocarditis through antiplatelet and antibacterial effects. *Circulation.* 1999;99:2791–2797.
515. Chan KL, Dumesnil JG, Cujej B, et al. A randomized trial of aspirin on the risk of embolic events in patients with infective endocarditis. *J Am Coll Cardiol.* 2003;42:775–780.
516. Tornos P, Almirante B, Mirabet S, et al. Infective endocarditis due to *Staphylococcus aureus*: deleterious effect of anticoagulant therapy. *Arch Intern Med.* 1999;159:473–475.
517. Garcia-Cabreria E, Fernandez-Hidalgo N, Almirante B, et al. Neurological complications of infective endocarditis: risk factors, outcome, and impact of cardiac surgery: a multicenter observational study. *Circulation.* 2013;127:2272–2284.
518. Dickerman S, Abrutyn E, Barsic B, et al. The relationship between the initiation of antimicrobial therapy and the incidence of stroke in infective endocarditis: an analysis from the ICE Prospective Cohort Study (ICE-PCS). *Am Heart J.* 2007;154:1086–1094.
519. Thuny F, Di Salvo G, Belliard O, et al. Risk of embolism and death in infective endocarditis: prognostic value of echocardiography: a prospective multicenter study. *Circulation.* 2005;112:69–75.
520. Sonneveld R, Mirabel M, Hajage D, et al. Neurologic complications and outcomes of infective endocarditis in critically ill patients: the ENDOCARDite in REAnimation prospective multicenter study. *Crit Care Med.* 2011;39:1474–1481.
521. Duval X, Hung B, Klein I, et al. Effect of early cerebral magnetic resonance imaging on clinical decisions in infective endocarditis: a prospective study. *Ann Intern Med.* 2010;152:497–504, W175.
522. Thuny F, Grisolí D, Collart F, et al. Management of infective endocarditis: challenges and perspectives. *Lancet.* 2012;379:965–975.
523. Sousa C, Botelho C, Rodrigues D, et al. Infective endocarditis in intravenous drug abusers: an update. *Eur J Clin Microbiol Infect Dis.* 2012;31:2905–2910.
524. Miro JM, Moreno A, Mestres CA. Infective endocarditis in intravenous drug abusers. *Curr Infect Dis Rep.* 2003;5:307–316.
525. Ribera E, Gomez-Jimenez J, Cortes E, et al. Effectiveness of cloxacillin with and without gentamicin in short-term therapy for right-sided *Staphylococcus aureus* endocarditis. A randomized, controlled trial. *Ann Intern Med.* 1996;125:969–974.
526. Heldman AW, Hartert TV, Ray SC, et al. Oral antibiotic treatment of right-sided staphylococcal endocarditis in injection drug users: prospective randomized comparison with parenteral therapy. *Am J Med.* 1996;101:68–76.
527. Al-Omari A, Cameron DW, Lee C, et al. Oral antibiotic therapy for the treatment of infective endocarditis: a systematic review. *BMC Infect Dis.* 2014;14:140.
528. Dzupova O, Roszypal H, Prochazka B, et al. Acute bacterial meningitis in adults: predictors of outcome. *Scand J Infect Dis.* 2009;41:348–354.
529. Aguilar J, Urday-Cornejo V, Donabedian S, et al. *Staphylococcus aureus* meningitis: case series and literature review. *Medicine (Baltimore).* 2010;89:117–125.
530. Pintado V, Meseguer MA, Fortun J, et al. Clinical study of 44 cases of *Staphylococcus aureus* meningitis. *Eur J Clin Microbiol Infect Dis.* 2002;21:864–868.
531. Pedersen M, Benfield TL, Skinhoej P, et al. Haemogenous *Staphylococcus aureus* meningitis. A 10-year nationwide study of 96 consecutive cases. *BMC Infect Dis.* 2006;6:49.
532. Pintado V, Pazos R, Jimenez-Mejias ME, et al. Methicillin-resistant *Staphylococcus aureus* meningitis in adults: a multicenter study of 86 cases. *Medicine (Baltimore).* 2012;91:10–17.
533. Grewal S, Hocking G, Wildsmith JA. Epidural abscesses. *Br J Anaesth.* 2006;96:292–302.
534. Self WH, Wunderink RG, Williams DJ, et al. *Staphylococcus aureus* community-acquired pneumonia: prevalence, clinical characteristics, and outcomes. *Clin Infect Dis.* 2016;63:300–309.
535. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med.* 2015;373:415–427.
536. Aliberti S, Reyes LF, Faverio P, et al. Global initiative for methicillin-resistant *Staphylococcus aureus* pneumonia (GLIMP): an international, observational cohort study. *Lancet Infect Dis.* 2016;16:1364–1376.
537. Gillet Y, Vanhems P, Lina G, et al. Factors predicting mortality in necrotizing community-acquired pneumonia caused by *Staphylococcus aureus* containing Panton-Valentine leukocidin. *Clin Infect Dis.* 2007;45:315–321.
538. Meyer CN, Rosenlund S, Nielsen J, et al. Bacteriological aetiology and antimicrobial treatment of pleural empyema. *Scand J Infect Dis.* 2011;43:165–169.
539. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis.* 2007;44 Suppl 2:S27–S72.
540. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis.* 2011;52:e18–e55.
541. Davis JS. Management of bone and joint infections due to *Staphylococcus aureus*. *Intern Med J.* 2005;35 Suppl 2:S79–S96.
542. Kremers HM, Nwojo ME, Ransom JE, et al. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. *J Bone Joint Surg Am.* 2015;97:837–845.
543. Murillo O, Grau I, Lora-Tamayo J, et al. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. *Clin Microbiol Infect.* 2015;21:254, e251–e258.
544. Zimmerli W. Clinical practice. Vertebral osteomyelitis. *N Engl J Med.* 2010;362:1022–1029.
545. White M, Dennison WM. Acute hematogenous osteitis in childhood: a review of 212 cases. *J Bone Joint Surg Br.* 1952;34-B:608–623.
546. Grammatico-Guillou L, Baron S, Gettner S, et al. Bone and joint infections in hospitalized patients in France, 2008: clinical and economic outcomes. *J Hosp Infect.* 2012;82:40–48.
547. Blyth MJ, Kincaid R, Craigen MA, et al. The changing epidemiology of acute and subacute haematogenous osteomyelitis in children. *J Bone Joint Surg Br.* 2001;83:99–102.
548. Dartnell J, Ramachandran M, Katchburian M. Haemogenous acute and subacute paediatric osteomyelitis: a systematic review of the literature. *J Bone Joint Surg Br.* 2012;94:584–595.
549. Lew DP, Waldvogel FA. Osteomyelitis. *Lancet.* 2004;364:369–379.
550. Peschel A, Otto M. Phenol-soluble modulins and staphylococcal infection. *Nat Rev Microbiol.* 2013;11:667–673.
551. Loughran AJ, Gaddy D, Beenken KE, et al. Impact of sarA and Phenol-Soluble Modulins on the Pathogenesis of Osteomyelitis in Diverse Clinical Isolates of *Staphylococcus aureus*. *Infect Immun.* 2016;84:2586–2594.
552. Cassat JE, Hammer ND, Campbell JP, et al. A secreted bacterial protease tailors the *Staphylococcus aureus* virulence repertoire to modulate bone remodeling during osteomyelitis. *Cell Host Microbe.* 2013;13:759–772.
553. Butalia S, Palda VA, Sergeant RJ, et al. Does this patient with diabetes have osteomyelitis of the lower extremity? *JAMA.* 2008;299:806–813.
554. Proctor RA, Kriegeskorte A, Kahl BC, et al. *Staphylococcus aureus* Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections. *Front Cell Infect Microbiol.* 2014;4:99.
555. Peltola H, Paakkonen M, Kallio P, et al. Osteomyelitis-Septic Arthritis Study G. Short- versus long-term antimicrobial treatment for acute hematogenous osteomyelitis of childhood: prospective, randomized trial on 131 culture-positive cases. *Pediatr Infect Dis J.* 2010;29:1123–1128.
556. Guglielmo BJ, Luber AD, Paletta D Jr, et al. Ceftriaxone therapy for staphylococcal osteomyelitis: a review. *Clin Infect Dis.* 2000;30:205–207.
557. Tice AD, Hoagland PA, Shoultz DA. Risk factors and treatment outcomes in osteomyelitis. *J Antimicrob Chemother.* 2003;51:1261–1268.
558. Berbari EF, Kanj SS, Kowalski TJ, et al. 2015 Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and treatment of native vertebral osteomyelitis in adults. *Clin Infect Dis.* 2015;61:e26–e46.
559. Russell CD, Ramaesh R, Kalima P, et al. Microbiological characteristics of acute osteoarticular infections in children. *J Med Microbiol.* 2015;64(Pt 4):446–453.

560. Geirsson AJ, Statkevicius S, Vikingsson A. Septic arthritis in Iceland 1990–2002: increasing incidence due to iatrogenic infections. *Ann Rheum Dis.* 2008;67:638–643.
561. Lieber SB, Fowler ML, Zhu C, et al. Clinical characteristics and outcomes of septic bursitis. *Infection.* 2017;45:781–786.
562. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. *Swiss Med Wkly.* 2005;135:243–251.
563. Sendi P, Banderet F, Gruber P, et al. Periprosthetic joint infection following *Staphylococcus aureus* bacteraemia. *J Infect.* 2011;63:17–22.
564. Lalueza A, Morales-Cartagena A, Chaves F, et al. Risk factors for metastatic osteoarticular infections after a long follow-up of patients with *Staphylococcus aureus* bacteraemia. *Clin Microbiol Infect.* 2015;21:1010, e1011–e1015.
565. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med.* 2004;351:1645–1654.
566. Bickels J, Ben-Sira L, Kessler A, et al. Primary pyomyositis. *J Bone Joint Surg Am.* 2002;84-A:2277–2286.
567. Garcia C, Hallin M, Deplano A, et al. *Staphylococcus aureus* causing tropical pyomyositis, Amazon Basin, Peru. *Emerg Infect Dis.* 2013;19:123–125.