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# Staphylococcal Infections

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Staphylococcal disease has been recognized in neonates for centuries and reported at least as early as 1773, when pemphigus neonatorum was described.<sup>1</sup> Outbreaks of staphylococcal disease in nurseries were first noted in the late 1920s,<sup>2</sup> and the memorable term “cloud baby” was subsequently coined to describe index cases, often asymptomatic, who contaminated the nursery atmosphere with *Staphylococcus aureus* that colonized their respiratory tract, skin, or umbilical cord.<sup>3</sup> Until the late 1970s, staphylococcal disease in newborn infants was caused most often by *S. aureus*.<sup>4</sup> However, in the recent decades, coagulase-negative staphylococci (CoNS) have assumed an equally important role, especially in premature infants in neonatal intensive care units (NICUs),<sup>5-7</sup> often responsible for half or more of all cases of clinically significant bacterial disease. Management of staphylococcal disease in infants has become increasingly more complicated, reflecting the increasing incidence of methicillin resistance and the threat of vancomycin resistance among isolates of *S. aureus* and CoNS. This chapter summarizes current information about *S. aureus* and CoNS and the diseases these organisms produce in newborns and young infants.

## Epidemiology and Transmission

### STAPHYLOCOCCUS AUREUS

Many factors influence transmission of staphylococci among newborns, including nursery design, density of the infant population, and obstetric and nursing practices. Other factors influencing transmission include virulence properties of the individual *S. aureus* strains and immunogenetic factors characteristic of the newborn host. A particular factor that is critical in one epidemic may not be a driving factor under different circumstances.

Quantitative culture studies demonstrate that very small numbers of *S. aureus* are capable of establishing colonization in the newborn. Fewer than 10 bacteria can initiate umbilical colonization in 50% of newborns, whereas approximately 250 organisms can produce the same effect on the nasal mucosa.<sup>8</sup> Colonization of the newborn umbilicus, nares, and skin takes place early in life. By the fifth day in the nursery, the colonization rate among nursery inhabitants may be as high as 90%.<sup>9</sup> The umbilicus or rectum usually is colonized before the nares are.<sup>10,11</sup>

Most evidence indicates that the initial and perhaps major source of *S. aureus* infection is medical and nursing personnel.<sup>8</sup> A *S. aureus* strain common among medical attendants is far more likely than a maternal strain to colonize a given infant in the nursery<sup>12</sup>; in 85% of cases, infant colonization with *S. aureus* is likely to originate from an attendant's touch.<sup>13</sup> Persons with overt cutaneous lesions or disease often are highly contagious, but asymptomatic carriers can also be contagious,<sup>14</sup> and carriage on the skin, in the anterior nares, and in the perineal area is relevant.<sup>15,16</sup> The frequency of intestinal carriage of the pathogen may be greatly underestimated.<sup>17</sup>

Soon after the introduction of methicillin in 1960, methicillin-resistant *S. aureus* (MRSA) emerged as an important nosocomial pathogen.<sup>18</sup> For MRSA, resistance is mediated through the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with markedly reduced affinity for most  $\beta$ -lactam antibiotics.<sup>19</sup> MRSA isolates frequently harbor additional antibiotic resistance determinants, further limiting treatment options. Risk factors for MRSA infection include prior antimicrobial treatment, prolonged hospitalization, and stay within an intensive care unit.<sup>20</sup> Since the mid-1990s, infection with community-acquired MRSA (CA-MRSA) has been reported increasingly in patients without hospital contact or traditional risk factors for MRSA.<sup>21,22</sup> CA-MRSA strains typically have a distinct antibiotic susceptibility pattern, and more frequently cause skin and soft tissue infections or necrotizing pneumonias compared with methicillin-susceptible *S. aureus* (MSSA). CA-MRSA isolates are readily transmitted between family members and close contacts.<sup>22</sup>

The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network reported that from 2006 to 2008, approximately 3.7% of initial episodes of late-onset sepsis (LOS) or meningitis among very-low-birth-weight (VLBW) infants (<1500 g) were caused by *S. aureus*, with 28% of these attributable to MRSA; outcomes were similar in the two groups.<sup>23</sup> Carey and colleagues<sup>24</sup> reported the epidemiology of MSSA and MRSA in the NICU at Columbia University Medical Center. During the study period, there were 123 infections caused by MSSA and 49 infections caused by MRSA (28%). Overall, the clinical presentations and the crude mortality rates (16%-17%) were similar in both groups, although infants with MRSA infections were significantly younger at clinical presentation than infants with MSSA infections. The most common manifestations were bacteremia (36%), skin/soft tissue/wound infection (31%), bacteremia plus skin/soft tissue infection (15%), endocarditis (7%), and rare cases of tracheitis, osteomyelitis, meningitis, or mediastinitis. The risk of developing MSSA or MRSA infection was inversely related to birth weight, with 53% of infections occurring in VLBW infants and the majority of infections in infants weighing greater than 2500 g associated with surgical procedures. Reports of small outbreaks of community-acquired MRSA in NICUs and well-baby nurseries have appeared with increasing frequency.<sup>25-27</sup>

When clusters of staphylococcal disease associated with hospital exposure occur, temporal clustering of cases suggests the possibility of an outbreak caused by a single strain.<sup>28</sup> In these situations, strain identity requires characterization based on a molecular technique, such

as pulsed-field gel electrophoresis (PFGE) or multilocus sequence typing (MLST). MLST is a sequence-based typing system that uses the sequence of seven or more housekeeping genes to evaluate the genetic relatedness of strains of staphylococci.<sup>29</sup> The discriminatory power of this approach is less than that of PFGE, so the usefulness for the evaluation of local outbreaks is less.<sup>30</sup> Next-generation whole-genome sequencing has recently been applied to investigate MRSA transmission in the NICU setting and identified an increased risk of transmission from infants with as yet undiscovered MRSA colonization, in contrast to known MRSA-positive infants.<sup>31</sup>

## COAGULASE-NEGATIVE STAPHYLOCOCCI

Coagulase-negative staphylococci are common inhabitants of human skin and mucous membranes. *Staphylococcus epidermidis* is the species found most commonly as a member of the normal flora of the nasal mucosa and the umbilicus of the newborn.<sup>32</sup> With sensitive culture techniques, the nose, umbilicus, and chest skin are colonized with CoNS in up to 83% of neonates by 4 days of age.<sup>33</sup> Rates of colonization with *S. epidermidis* in one study of infants in a large academic NICU were as follows: nose, 89%; throat, 84%; umbilicus, 90%; and stool, 86%; simultaneous percentages for *S. aureus* were 17%, 17%, 21%, and 10%, respectively.<sup>32</sup> Although most infants acquire CoNS from environmental sources, including hospital personnel, a small percentage are colonized by vertical transmission.<sup>34,35</sup> Isolates of *S. epidermidis* and other CoNS resistant to multiple antibiotic agents are common. In a study involving premature neonates, D'Angio and associates<sup>36</sup> demonstrated that the incidence of strains resistant to multiple antibiotics rose from 32% to 82% by the end of the first week of life.

The observation that CoNS are important nosocomial pathogens among newborns, especially low-birth-weight (LBW) infants in NICUs, is explained by the prevalence of colonization with these organisms at multiple sites and the widespread use of invasive therapeutic modalities that subvert normal host epithelial barrier defenses. Examples of invasive treatments include endotracheal intubation, mechanical ventilation, placement of umbilical and other central venous catheters, urinary bladder catheters, and ventriculoperitoneal shunts, and the use of feeding tubes. In recent epidemiology, CoNS account for greater than half of bloodstream isolates obtained from neonates with LOS.<sup>5-7</sup> An inverse relationship exists between the rate of infection with CoNS and both birth weight and gestational age. Additional risk factors that are associated with CoNS bacteremia among VLBW neonates include respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, severe intraventricular hemorrhage, and necrotizing enterocolitis (NEC).<sup>5,7</sup>

Certain nutritional factors are associated with the development of LOS, including delayed initiation of enteral feeding, prolonged period to reach full enteral feeding status, delayed re-attainment of birth weight, and prolonged parenteral hyperalimentation.<sup>5</sup> Administration of intralipids through a Teflon catheter has also been shown in a case-control study to be associated with an increased risk of bacteremia caused by CoNS.<sup>37</sup> Most experts feel the clinical and experimental data suggest that CoNS have not become more virulent over time. Rather, these ubiquitous

organisms have become more common pathogens because therapeutic approaches have become increasingly invasive and because VLBW premature infants with compromised immunity are surviving longer. Indeed, the very interventions sustaining the hospitalized LBW neonate concurrently expose them to serious nosocomial infections, with CoNS at the forefront of causative agents.<sup>7</sup>

## Microbiology

Staphylococci are members of the family Micrococcaceae and are nonmotile, non-spore-forming bacteria that are catalase-negative. Species of staphylococci are separated into two large groups on the basis of ability to produce the extracellular enzyme coagulase. Organisms that produce coagulase are known as coagulase-positive staphylococci, primarily *S. aureus*,<sup>38</sup> and organisms that produce no coagulase are referred to as CoNS. The presence of coagulase can be evaluated either by assessing broth medium for secreted enzyme, which reacts with coagulase-reacting factor in plasma and results in formation of a fibrin clot, or by testing for cell-bound enzyme, which results in clumping when a suspension of organisms is incubated with plasma.

Staphylococci grow best in an aerobic environment but can proliferate under anaerobic conditions as well. They grow readily on most routine laboratory media, including Luria broth, and usually are isolated from clinical specimens by using sheep blood agar. Gram stain reveals gram-positive cocci ranging from 0.7 to 1.2  $\mu\text{m}$  in diameter, usually visible in irregular grapelike clusters (Fig. 14-1A). Growth in liquid culture often results in a predominance of single cocci, pairs, tetrads, and chains of three or four cells. Of note, dying organisms and bacteria in stationary phase or ingested by phagocytes may appear to be gram-negative. Growth on blood agar results in round, convex, shiny opaque colonies that are 1 to 2 mm in diameter after 24 hours of incubation. *S. aureus* colonies often are deep yellow or golden in color and typically are surrounded by a zone of  $\beta$ -hemolysis (see Fig. 14-1B). By contrast, colonies of CoNS usually are chalk-white, often lacking surrounding hemolysis.

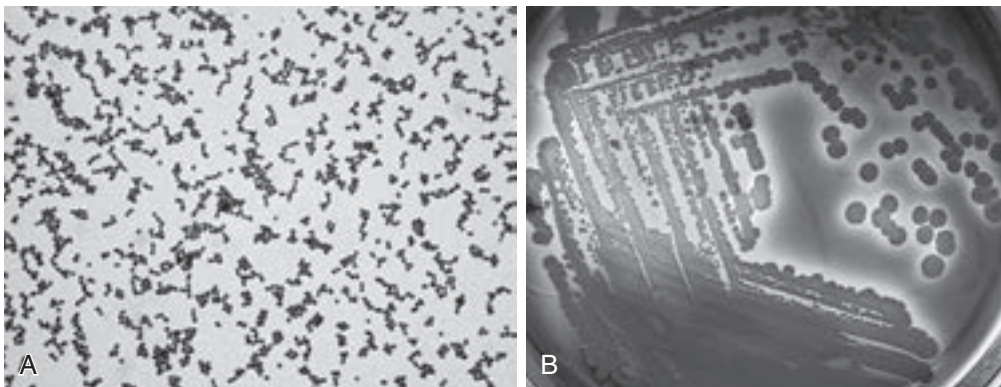
### STAPHYLOCOCCUS AUREUS

The staphylococcal cell wall is composed of two major components, peptidoglycan and teichoic acid.<sup>39,40</sup> *S. aureus* peptidoglycan is composed of chains of N-acetylglucosamine,

N-acetylmuramic acid, alanine, glutamic acid, and lysine or diaminopimelic acid, with pentaglycine bridges that cross-link these chains. Four transpeptidases, or penicillin-binding proteins called PBP1, PBP2, PBP3, and PBP4 play an important role in peptidoglycan biosynthesis and are inactivated by  $\beta$ -lactams.<sup>41</sup> A mutated form of PBP2 (PBP2a) encoded by the *mecA* gene is the basis of methicillin resistance in the current epidemic of hospital- and community-acquired MRSA. Teichoic acid is a polymer of ribitol phosphate that is held in the cell wall by covalent attachment to the insoluble peptidoglycan. Staphylococcal teichoic acid is antigenic, and antibodies to this substance cause agglutination of isolated staphylococcal cell walls.<sup>42</sup> Antibodies to teichoic acid enhance opsonophagocytic killing of nonencapsulated strains of *S. aureus* but have little effect on encapsulated isolates.<sup>43</sup> By way of contrast, antibodies to peptidoglycan play a key role in the opsonization of encapsulated *S. aureus*.<sup>44</sup> Antibodies to both *S. aureus* teichoic acid and peptidoglycan are widespread in screens of the human population.<sup>44</sup>

In addition to peptidoglycan and teichoic acid, other components of the *S. aureus* cell wall include protein A, an immunoglobulin Fc-binding protein, and a number of other surface-expressed proteins. Similar to the situation with other gram-positive bacteria, many *S. aureus* proteins anchored in the cell wall possess a carboxy-terminal LP(X) TG motif, which serves as a sorting signal for a membrane enzyme called sortase (SrtA).<sup>45,46</sup> This enzyme cleaves polypeptides between the threonine and the glycine of the LP(X) TG motif and catalyzes formation of an amide bond between the carboxy group of threonine and the amino group of peptidoglycan cross-bridges.<sup>46</sup> These include several proteins involved in extracellular matrix binding and promoting *S. aureus* adherence to host epithelium.<sup>47</sup>

*S. aureus* produces a polysaccharide capsular layer external to the cell wall. Capsular antigens are limited in antigenic specificity and highly conserved among clinical isolates, where the predominant capsules identified are serotype 5 and serotype 8.<sup>48</sup> The serotype 5 *S. aureus* capsule has the structure  $(\rightarrow 4)\text{-}3\text{-}O\text{-}Ac\text{-}\beta\text{-}D\text{-}ManNAcA\text{-}(1\rightarrow 4)\text{-}\alpha\text{-}l\text{-}FucNAc\text{-}(1\rightarrow 3)\text{-}\beta\text{-}D\text{-}FucNAc\text{-}(1\rightarrow)_n$ , while serotype 8 capsule has the structure  $(\rightarrow 3)\text{-}4\text{-}O\text{-}Ac\text{-}\beta\text{-}D\text{-}ManNAcA\text{-}(1\rightarrow 3)\text{-}\alpha\text{-}l\text{-}FucNAc\text{-}(1\rightarrow 3)\text{-}\beta\text{-}D\text{-}FucNAc\text{-}(1\rightarrow)_n$ .<sup>49,50</sup> Although these two capsular polysaccharides differ only in the sugar linkages the sites of *O*-acetylation of the mannosaminuronic acid residues, they remain serologically distinct. Capsule plays a role in the pathogen's resistance to phagocyte



**Figure 14-1** **A**, Gram stain of *Staphylococcus aureus* showing characteristic clusters. **B**, Blood-agar plate showing growth of *S. aureus* with zone of  $\beta$ -hemolysis surrounding colonies.

clearance.<sup>48</sup> Loss of capsule expression may be associated with bacterial persistence during chronic infections.<sup>51</sup>

Small colony variants (SCVs) of *S. aureus* isolated from clinical specimens have been recognized for nearly a century. SCVs have now been linked to persistent and relapsing *S. aureus* infections, including chronic osteomyelitis and soft tissue abscesses.<sup>52,53</sup> These phenotypes can be traced to biochemical defects in electron transport, which are associated with the slow growth and reduced  $\alpha$ -toxin production and which promote survival and persistence within endothelial cells. It is hypothesized that the intercellular location represents a privileged niche against the actions of host innate defense molecules and antibiotics.<sup>54</sup> Because they can be overlooked in the laboratory because of their fastidious growth, extra efforts to identify SCVs should be undertaken in the setting of persistent or relapsing *S. aureus* infection despite antibiotic therapy.<sup>52,53</sup>

Nucleotide sequencing of the whole genome for several isolates of *S. aureus*, including MRSA strains,<sup>55,56</sup> have established that the genome is 2.8 to 2.9 Mb in size, with approximately 2600 to 2700 open reading frames (ORFs) and an overall guanine:cytosine content of approximately 33%.<sup>57,58</sup> Much of the *S. aureus* genome appears to have been acquired by lateral gene transfer.<sup>55</sup> Most antibiotic resistance genes are carried on mobile genetic elements, including a unique resistance island. Pathogenicity islands belonging to at least three different classes have been identified, including toxic shock syndrome (TSS) toxin islands, exotoxin islands, and enterotoxin islands. Of interest, the exotoxin and enterotoxin islands are closely linked to other gene clusters encoding putative virulence factors. Historically, phage typing and serologic typing were the most common systems for differentiating strains of *S. aureus* for epidemiologic purposes.<sup>59</sup> Later, molecular approaches such as PFGE and MLST became the standard for defining strain identity in a patient with multiple isolates or in a possible outbreak involving multiple patients.<sup>60,61</sup> Next-generation high-throughput genotyping technologies are now becoming the standard to understand the geographic origin and intrahospital spread of important microbial pathogens such as *S. aureus*,<sup>62</sup> including the analysis of NICU outbreaks.<sup>31</sup>

## COAGULASE-NEGATIVE STAPHYLOCOCCI

Coagulase-negative staphylococci are a heterogeneous group of organisms divided into 32 species.<sup>38</sup> The following 15 species of CoNS are found as members of the normal human flora: *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. capitis*, *S. warnerii*, *S. hominis*, *S. xylosum*, *S. cohnii*, *S. simulans*, *S. auricularis*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. lugdunensis*, and *S. schleiferi*.<sup>38,63</sup> Among these species, several occupy very specific niches on the skin. For example, *S. capitis* is most abundant on the head, where sebaceous glands are plentiful. *S. auricularis* has a striking predilection for the external auditory canal. *S. hominis* and *S. haemolyticus* are most common in the axillae and the pubic area, where apocrine glands are numerous.

Speciation of CoNS is accomplished on the basis of a series of biochemical characteristics, simplified in recent years by the commercial availability of available miniaturized kits.<sup>38</sup> Differentiation of two strains belonging to the same species (subspeciation) represents a more difficult problem,

however. Analogous to the situation with *S. aureus*, techniques for distinguishing strains of a given species include PFGE, MLST, and next-generation sequencing.<sup>64,65</sup> The composition of CoNS is quite similar to the makeup of *S. aureus*, except that the teichoic acid contains glycerol in place of ribose and the cell wall lacks protein A. Determination of the genome of *S. epidermidis* strain ATCC 12228 (a commensal isolate not associated with disease) revealed a genome approximately 2.5 Mb in size, with 2419 ORFs, greater than 10% smaller than the published genomes of *S. aureus* isolates.<sup>66</sup> In comparison with the available *S. aureus* genomes, ATCC 12228 contains fewer antibiotic resistance genes and lacks pathogenicity islands and a capsule locus. A homologue of the *S. aureus* *srtA* gene is present, along with nine proteins predicted to contain an LP(X)TG motif.

## Pathogenesis of Disease

### VIRULENCE MECHANISMS OF STAPHYLOCOCCUS AUREUS

The pathogenic process of *S. aureus* infection begins with colonization of host skin or mucosal surfaces and involves bacterial attachment to host cells, often via components of the extracellular matrix. To persist, the organism produces molecules that decrease the effectiveness of complement and antibody-mediated opsonophagocytosis and block effectors of host immune cell killing, such as reactive oxygen species and antimicrobial peptides. Ultimately, the organism expresses specific factors that damage host cells and degrade components of the extracellular matrix, thus contributing to persistence and facilitating spread within normally sterile sites of the host.

### Epithelial Attachment and Invasion

*S. aureus* initiated adherence by binding to components of the extracellular matrix of the host. This adherence is mediated by protein adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which are typically covalently anchored to the cell wall peptidoglycan through the action of sortase enzymes that recognize an LP(X)TG motif in the C-terminal region of the protein.<sup>67,68</sup> *S. aureus* MSCRAMMs can promote binding to fibronectin, fibronogen, and collagen. Most strains express two related fibronectin-binding proteins, FnBPA and FnBPB, which mediate bacterial attachment to immobilized fibronectin in vitro and contribute to *S. aureus* binding to plasma clots and foreign bodies removed from the host.<sup>69</sup> *S. aureus* also expresses the fibrinogen-binding proteins, or “clumping factors” ClfA and ClfB.<sup>70</sup> Each Clf protein recognizes a different part of the fibrinogen model, and together, may synergistically act to allow *S. aureus* to attach more firmly to vascular thrombi under flow stress within the bloodstream. A fibronectin bridge from surface-anchored *S. aureus* ClfA to integrins in the epithelial cell surface promotes intracellular invasion by the pathogen.<sup>71</sup> In rat endocarditis studies, ClfA mutant *S. aureus* strains have reduced virulence.<sup>72</sup> Finally, the collagen-binding MSCRAMM Cna allows *S. aureus* to adhere to collagenous tissues such as cartilage<sup>73</sup>; a Cna-null mutant strain of *S. aureus* is significantly attenuated

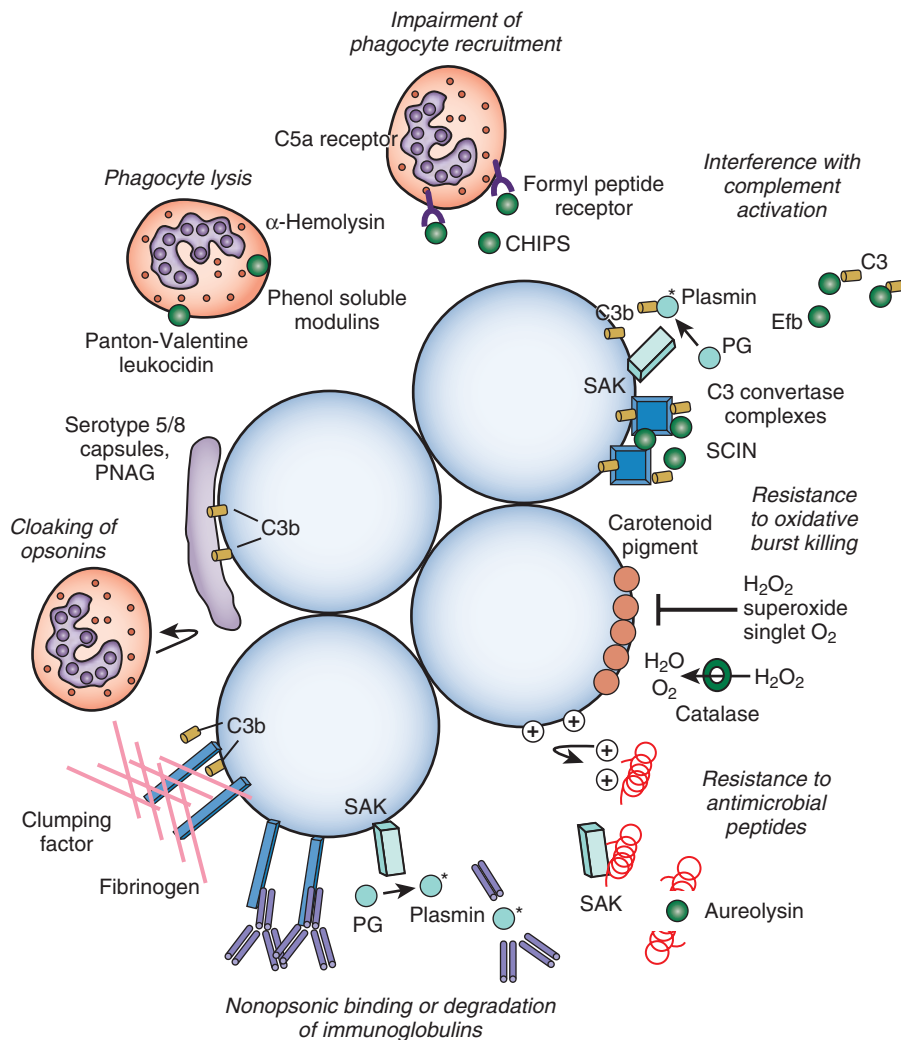
for virulence in a murine septic arthritis model.<sup>74</sup> The *icaADBC*-encoded polysaccharide intercellular adhesin (PIA) or polymeric *N*-acetyl-glucosamine (PNAG) contributes to *S. aureus* biofilm development<sup>75</sup>; these genes and a resultant phenotype shared by *S. epidermidis* are discussed in more detail later.

### Innate Immune Resistance

The propensity of *S. aureus* to produce systemic infections, even in otherwise healthy infants, children, and adults, reflects the capacity of this pathogen to resist host innate immune clearance mechanisms that normally function to prevent microbial dissemination beyond epithelial surfaces. The multiple mechanisms used by this preeminent disease agent are summarized schematically in Figure 14-2.

Cationic antimicrobial peptides, such as cathelicidins and defensins, produced by epithelial cells and phagocytes are

an important first line of defense against invasive bacterial infection. By incorporating positively charged residues into its cell wall lipoteichoic and teichoic acid, *S. aureus* increases electrostatic repulsion of these defense peptides. D-alanylation of teichoic acids mediated by the *dlt* operon is present in both pathogens, promoting resistance to adenosine monophosphate and neutrophil killing by increasing surface charge.<sup>76,77</sup> In addition, positively-charged lysyl-phosphatidylglycerol modifications of teichoic acids are encoded in the functions of the *S. aureus* *mprF* or *lysC* genes, and contribute to human antimicrobial peptide resistance.<sup>78,79</sup> *S. aureus* mutants defective in *dlt* or *mprF* genes show reduced virulence in small animal infection models.<sup>77,80</sup> The secreted proteases V8 and aureolysin of *S. aureus* function to degrade antimicrobial peptides, which could contribute further to *S. aureus* resistance to this important branch of the innate defense system.<sup>81,82</sup>



**Figure 14-2** *Staphylococcus aureus* possesses multiple virulence mechanisms to resist clearance by host phagocytic cells. Phagocyte recruitment is restricted by chemotaxis inhibitory protein of staphylococci (CHIPS) binding to chemokine receptors. Complement activation is blocked by protein Efb binding of soluble C3 and inhibition of the both the classical/lectin and alternative C3 convertases by staphylococcal complement inhibitor (SCIN). Staphyloxanthin pigment provides an antioxidant shield, whereas catalase detoxifies hydrogen peroxide ( $H_2O_2$ ). Resistance to cationic antimicrobial peptides is afforded by positive charge modifications of the cell wall, aureolysin-mediated proteolysis, and binding/inactivation by staphylokinase (SAK). Protein A binds the Fc domain of immunoglobulin G in a nonopsonic manner, whereas fibrinogen-binding clumping factor and the surface polysaccharide capsule and poly-*N*-acetylglucosamine (PNAG) cloak surface-bound opsonins from phagocyte recognition. The heptameric pore-forming toxins  $\alpha$ -hemolysin and Panton-Valentine leukocidin and phenol-soluble modulins target leukocyte membranes. The plasminogen (PG)-binding protein staphylokinase (SAK) activates the zymogen to the active protease plasmin, which can degrade complement opsonin C3b and the immunoglobulin Fc domain.

Many *S. aureus* strains produce the chemotaxis inhibitory protein of staphylococci (CHIPS), which binds with high avidity to the leukocyte receptors for C5a and N-formyl peptides, thereby blocking functional engagement of the respective chemoattractants, and delaying neutrophil recruitment to the site of infection.<sup>83</sup> *S. aureus* also expresses the extracellular adherence protein (Eap), which binds and inhibits intercellular adhesion molecule-1 (ICAM-1), the endothelial receptor required to initiate leukocyte adhesion and diapedesis.<sup>84</sup>

*S. aureus* expresses multiple factors to interfere with host complement-mediated clearance.<sup>85</sup> Cleavage of C3 to opsonically active C3b is accomplished after assembly of C3 convertase complexes: C4bC2a (classical/lectin pathways) or C3bBb (alternative pathway) on the bacterial surface. The secreted approximately 10-kD *S. aureus* protein known as staphylococcal complement inhibitor (SCIN) binds and stabilizes both convertases on the bacterial surface, preventing generation of additional convertases, impairing their enzymatic activities, and effectively inhibiting all three complement pathways.<sup>86</sup> *S. aureus* clumping factor binds the host complement inhibitory protein factor 1, which subsequently cleaves C3b into iC3b,<sup>87</sup> preventing further amplification of the complement cascade or its activation via the alternative pathway. The secreted *S. aureus* fibrinogen-binding protein Efb-C can bind free C3, altering the solution conformation of this critical complement component such that it is unable to participate in its downstream opsonization functions.<sup>88</sup> Finally, another mechanism of interference with complement opsonization derives from bacterial cooptation of host proteolytic activities. The *S. aureus* surface receptor staphylokinase binds plasminogen from host serum and converts the zymogen to the active protease plasmin. Surface-bound plasmin can then cleave human C3b and C3bi from the bacterial cell wall and impair neutrophil phagocytosis.<sup>89</sup> *S. aureus* possesses several broad-spectrum proteases, including the serine protease V8, which is capable of degrading C3a, C3b, C5a, and C5b,<sup>90</sup> and aureolysin, a metalloprotease that cleaves C3,<sup>91</sup> among other substrates.

*S. aureus* is able to inhibit effector function of immunoglobulin when the pathogen binds its Fc region, effectively decorating the bacterial surface with the host molecule in a “backwards,” nonopsonic orientation.<sup>92</sup> Most notably, protein A, the most abundant protein on the surface of *S. aureus*, binds to the Fc region of IgM and IgG to inhibit opsonophagocytic killing.<sup>93</sup> In addition, the majority of *S. aureus* clinical isolates express surface capsules composed of serotype 5 or 8 polysaccharide.<sup>48</sup> The presence of *S. aureus* capsule is associated with reduced opsonophagocytic uptake of the pathogen by neutrophils and increased virulence in a mouse bacteremia model.<sup>94,95</sup> Analogous functions can be ascribed to an additional *S. aureus* surface polysaccharide, poly-N-acetylglucosamine.<sup>96</sup> Neither of the *S. aureus* exopolysaccharides directly inhibit deposition of complement factors on the bacterial surface; rather they appear to serve as a superficial “cloak” that restricts access of phagocytes to the opsonins.<sup>92</sup>

Catalase production is a diagnostic tool used to distinguish staphylococci from streptococci in the clinical laboratory, and the ability of staphylococcal catalase to detoxify hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generated during oxidative burst

may promote phagocyte resistance and virulence.<sup>97</sup> The golden pigment for which *S. aureus* is named is a carotenoid molecule with potent antioxidant properties that are necessary and sufficient to promote bacterial neutrophil resistance and virulence in a subcutaneous infection model.<sup>98,99</sup> The superoxide-driven Fenton reaction between H<sub>2</sub>O<sub>2</sub> and an appropriate transition metal (e.g., iron) catalyst generates highly toxic hydroxyl radicals important in phagocyte killing. However, *S. aureus* expression of bacterial nitric oxide (NO) synthase generates NO that inhibits Fenton chemistry.<sup>100,101</sup> *S. aureus* also resists oxidative stress through superoxide dismutases, as confirmed by diminished *in vivo* survival of mutants lacking these enzymes.<sup>102</sup>

### Secreted Toxins

A number of *S. aureus* secreted factors possess cytolytic activity against host cells and can serve to facilitate tissue spread, promote inflammatory responses and, especially when the target is a phagocytic cell, promote bacterial innate immune evasion. Perhaps the best-studied is *S. aureus*  $\alpha$ -toxin (also referred to as  $\alpha$ -hemolysin), which forms heptamers in the membranes of a variety of cell types, creating large pores.<sup>103,104</sup> Pore formation induced by *S. aureus*  $\alpha$ -toxin is associated with release of nitric oxide from endothelial cells and stimulation of apoptosis in lymphocytes.<sup>105,106</sup> *S. aureus* production of  $\alpha$ -toxin may also promote escape from the phagolysosome after macrophage engulfment.<sup>107</sup> MRSA production of  $\alpha$ -toxin, which binds to and activates the metalloprotease ADAM-10 in lung epithelial cells, is associated with high lethality in the mouse model of pneumonia.<sup>108</sup> Immunization with an inactivated form of  $\alpha$ -toxin, which cannot form pores, generates antigen-specific immunoglobulin G responses and provides protection against MRSA pneumonia.<sup>109</sup>

*S. aureus* also produces an additional family of two-subunit heteroheptameric toxins capable of oligomerizing in the membrane of target leukocytes to produce pores and promote hypoosmotic cell lysis. These include  $\gamma$ -hemolysin, leukotoxin ED, and the bacteriophage-encoded Panton-Valentine leukocidin (PVL).<sup>110</sup> The  $\gamma$ -hemolysin is strongly upregulated during *S. aureus* growth in human blood<sup>111</sup> and opens calcium channels in neutrophil membranes,<sup>112</sup> inhibiting their oxidative burst.<sup>113</sup> Leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to deplete neutrophils<sup>114</sup> and CCR5 to damage other myeloid cell types and T cells.<sup>115</sup>

PVL has gained notoriety because of its strong epidemiologic association with severe cases of CA-MRSA infections.<sup>116</sup> Phage transduction of PVL into a previously naïve *S. aureus* background was reported to increase virulence in murine necrotizing pneumonia model,<sup>117</sup> but an inadvertent mutation in the accessory gene regulator (*agr*) locus of the test strain probably led to spurious interpretations of the PVL linkage to disease pathogenesis.<sup>118</sup> A more direct test of isogenic deletion of PVL in the epidemic USA300 and USA400 clones associated with severe CA-MRSA infections had no effect on neutrophil lysis nor virulence in murine skin abscess and systemic infection models<sup>119</sup> but did contribute to proinflammatory cytokine release and muscle necrosis at higher inoculums and in certain mouse genetic backgrounds.<sup>120</sup> Of note, mouse neutrophils are more resistant to PVL action than human or rabbit neutrophils.<sup>121</sup> In

a rabbit pneumonia model, PVL enhanced the capacity of USA300 MRSA to cause severe necrotic lung infection and death, and purified PVL administered directly into the lung caused severe lung injury by recruiting and lysing neutrophils, which caused lung damage by release of cytotoxic granule contents.<sup>122</sup>

Phenol-soluble modulins (PSMs) are a recently recognized group of small peptides produced by *S. aureus* with important roles in immune evasion and virulence.<sup>123</sup> PSMs possess an amphipathic  $\alpha$ -helical structure and membrane destabilizing properties that can recruit, activate, and lyse neutrophils by signaling through formyl peptide receptor-2,<sup>124</sup> inducing a marked proinflammatory response while promoting bacterial survival. PSMs allow *S. aureus* to escape the phagolysosome and replicate intracellularly<sup>125</sup> and can synergistically exaggerate the cytolytic effect of PVL on human neutrophils.<sup>126</sup> Other *S. aureus*-secreted toxins include  $\beta$ -hemolysin, a sphingomyelinase enzyme.<sup>127</sup> Through targeted mutagenesis,  $\beta$ -hemolysin was recently found to contribute to *S. aureus*-induced lung injury, neutrophilic inflammation, and vascular leakage of serum proteins into the alveolar spaces, in part mediated by the ability of the toxin to promote ectodomain shedding of syndecan-1, a major proteoglycan coating lung epithelial cells.<sup>128</sup>

*S. aureus* elaborates a number of toxins with superantigenic capacity, able to promote aberrant interaction between MHC class II on the surface of antigen-presenting cells (e.g., macrophages) with the  $\beta$ -chain of the T-cell receptor, leading to polyclonal T-cell activation and potentially staphylococcal TSS.<sup>129</sup> As many as 20 distinct *S. aureus* superantigens are known, prominently including TSS toxin-1, and staphylococcal enterotoxins A to E and G to J. The genes encoding the *S. aureus* superantigens are present on accessory genetic elements, such as prophages, transposons, plasmids, or chromosomal pathogenicity islands. The contribution of the superantigens to the severe disease manifestations of *S. aureus* is well demonstrated, but the potential evolutionary advantage of superantigen production to the pathogen is not clear. One possible advantage of T-cell activation at the site of infection might be dysregulated cytokine expression patterns that suppress effective local inflammatory responses.<sup>129</sup>

Certain strains of *S. aureus* express the exfoliative (epidermolytic) toxins ETA, ETB, ETC, and/or ETD. These toxins have recently been identified as glutamate-specific serine proteases that specifically and efficiently cleave a single peptide bond in the extracellular region of human and mouse desmoglein 1 (Dsg1), a desmosomal intercellular adhesion molecule, leading to the exfoliative phenotype of scalded skin syndrome and bullous impetigo.<sup>130,131</sup>

### Quorum Sensing and Regulation of Virulence Factor Expression

*S. aureus* appears to impose tight regulation on the differential expression of specific sets of virulence determinants at different stages of growth or the pathogenic process. For example, cell wall-associated adhesive factors that facilitate the initial stages of infection are selectively produced during the exponential phase of *in vitro* growth.<sup>132</sup> Conversely, almost all *S. aureus* extracellular proteins and secreted toxins presumed to play a greater role in evasion

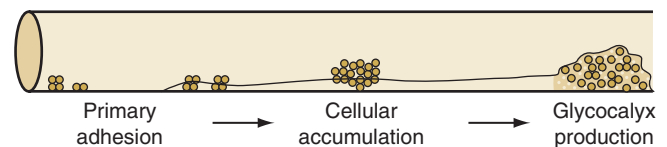
of the immune system and tissue spread are synthesized predominantly in the postexponential phase of growth.<sup>132</sup> These processes are under the cell-density (quorum sensing)-dependent control of the *agr* locus.<sup>133,134</sup> Like other bacterial quorum sensing systems, *agr* encodes an autoactivating peptide (AIP) that is the inducing ligand for the *agr* signal receptor (AgrC). The unique effector of global gene regulation in the *agr* system is the regulatory RNA molecule RNAPIII.<sup>134</sup> Agr mutants show decreased virulence in murine infection models.<sup>135</sup>

### VIRULENCE MECHANISMS OF COAGULASE-NEGATIVE STAPHYLOCOCCI

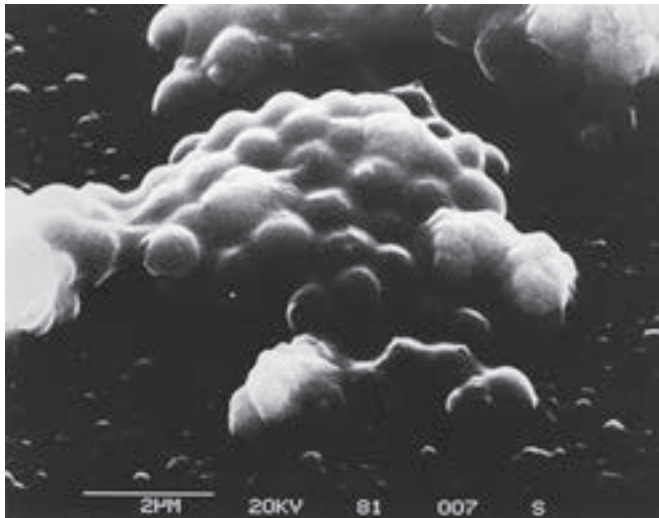
Until recently, the pathogenic potential of CoNS received little attention. With the emergence of these organisms as prominent pathogens in neonates and hospitalized patients with intravascular devices, investigation has intensified in an effort to identify important virulence factors and to inform new approaches to treatment and prevention.<sup>136</sup> Two main reasons for the increasing rate of CoNS infections are spreading antibiotic resistance among CoNS and the ever-increasing development and use of medical devices.<sup>137</sup> Attention has centered primarily on *S. epidermidis*, the species most commonly associated with clinical disease, usually in association with central intravenous catheters. Other species that have been examined, although to a lesser extent, include *S. saprophyticus*, *S. lugdunensis*, and *S. schleiferi*.

When CoNS infections are initiated on intravascular catheters and other prosthetic devices, the ability of the bacterium to adhere to the hydrophobic surface of the foreign body is a first critical step in the pathogenic process (Fig. 14-3). CoNS are able to colonize virtually any plastic surface.<sup>138</sup> In addition, plastic objects in the human body soon become coated with host extracellular matrix proteins,<sup>139</sup> such that CoNS can colonize the devices either by direct attachment to the plastic or by binding to the host extracellular matrix, and both processes are likely to play a role in the initial establishment of infection. Overall surface hydrophobicity varies among CoNS strains, and increased hydrophobicity can be correlated to better plastic binding<sup>140</sup>; however, no linkage between surface hydrophobicity and clinical infectivity has been established.<sup>137</sup>

Transposon mutagenesis identified AltE, a putative CoNS autolysin protein, as promoting adherence to plastic surfaces<sup>141</sup>; an *S. epidermidis* AltE mutant shows diminished pathogenicity in a rat model of catheter-associated infection.<sup>142</sup> Two large surface proteins present in some *S. epidermidis* strains, SSP-1 and SSP-2—one likely a degradation product of the other—are present in fibrillar structures on the bacterial surface and promote binding to polystyrene.<sup>143</sup>



**Figure 14-3** Schematic model of the phases involved in *Staphylococcus epidermidis* biofilm formation.



**Figure 14-4** Scanning electron micrograph showing the presence of a *Staphylococcus epidermidis* biofilm on an explanted intravascular catheter. The biofilm is characterized by multilayered cell clusters embedded in an extracellular polysaccharide. (From von Eiff C, Peters G, Heilmann C: Pathogenesis of infections due to coagulase-negative staphylococci, *Lancet Infect Dis* 2:677–685, 2002.)

Following in the pattern of *S. aureus*, extracellular matrix binding surface components or MSCRAMMs are beginning to be well characterized in CoNS. The fibrinogen-binding protein Fbe (also known as SdrG) of *S. epidermidis* resembles *S. aureus* clumping factor, with the presence of multiple serine/aspartate repeat domains and a capacity to bind to the  $\beta$ -chain of the host matrix protein.<sup>144–146</sup> *S. epidermidis* cell wall teichoic acid enhances overall adherence to fibronectin, perhaps serving as a bridging molecule between bacterial MSCRAMMs and fibronectin-coated surfaces.<sup>147</sup> Phage-display technology was used to identify an *S. epidermidis* protein, EmbP, capable of mediating binding to fibronectin, whereas GehD, an *S. epidermidis* lipase enzyme, appears to promote collagen attachment.<sup>148</sup> Finally, the above-mentioned AltE also contains a domain with vitronectin-binding capacity that may contribute to its virulence phenotype in the rat model.<sup>141</sup>

After initial attachment to a biomaterial, organisms multiply and form complex multilayered aggregates that involve intercellular adhesion and are referred to as biofilms (Fig. 14-4; see also Fig. 14-3). Historically, isolates of CoNS were often described as elaborating “slime” and “slime-associated antigen,” terms that we now realize refer to biofilms and the presence of abundant quantities of a specific polysaccharide molecule. Significant degrees of slime production were reported in greater than 80% of CoNS isolates from infants with invasive disease.<sup>149,150</sup> The main virulence factor responsible for the formation of these cellular aggregates in certain *S. epidermidis* strains is now recognized to be a secreted exopolysaccharide called polysaccharide intercellular adhesin, or PIA.<sup>151,152</sup> PIA is an unbranched  $\beta$ -1,6-linked *N*-acetylglucosaminic acid polymer, produced by the enzymes of the four-gene *ica* operon.<sup>153</sup> An *ica* knockout mutant shows reduced virulence in a rat model of catheter infection,<sup>142</sup> and *S. epidermidis* strains isolated from patients with foreign infections were more likely to possess the *ica* genes and form robust biofilms *in vitro* than strains from

asymptomatic individuals.<sup>154</sup> Expression of the PIA is subject to on-off phase-switching that may be attributable to reversible insertion and excision of mobile genetic element (IS256) in the *ica* operon.<sup>155</sup> A 140-kD CoNS extracellular protein known as accumulation-associated protein (AAP) appears to cooperate with PIA in promoting biofilm growth.<sup>156</sup>

CoNS biofilm formation provides a nonspecific physical barrier to cellular and humoral defense mechanisms.<sup>152,157</sup> The formation of CoNS biofilms is dependent upon the regulatory control exerted by a homologue of the *S. aureus agr* locus.<sup>158</sup> *S. epidermidis* embedded within biofilms binds less complement C3b and IgG and is therefore less susceptible to neutrophil killing.<sup>159</sup> CoNS biofilm-associated polysaccharide also is capable of inhibiting the antimicrobial action of both vancomycin and teichoplanin.<sup>160</sup> In the clinical setting, formation of biofilms on the catheter surface has been shown to make eradication of CoNS infection more problematic.<sup>161,162</sup>

*S. epidermidis* expresses a 27-kD serine protease called GluSE that is expressed during biofilm formation and has been shown to degrade fibrinogen and the complement-derived chemoattractant C5, suggesting a potential role in immune evasion.<sup>163</sup> *S. epidermidis* also express a group of secreted amphiphilic peptides called phenol-soluble modulins (PSM), which have neutrophil chemotactic ability and generate other proinflammatory effects, including activating neutrophil oxidative burst and degranulation.<sup>164</sup>

## ROLE OF HOST DEFENSES

Even under the most ideal conditions, infants in the hospital are surrounded by staphylococci. Physical barriers such as the skin and mucous membranes represent a major defense against staphylococcal disease. Bacteremic disease most often develops when organisms colonizing the skin gain access to the bloodstream through the portal created by an intravascular catheter. Other routes for entry into the bloodstream include the intestinal tract after injury to the epithelial barrier, the respiratory tract in patients receiving mechanical ventilation, and the umbilicus when the umbilical cord remains in place. Newborn innate immunity demonstrates several distinct deficits predisposing to staphylococcal infection, including diminished skin integrity, impaired cytokine responses, low complement levels, and reduced expression of innate antimicrobial peptides, all of which are exaggerated in preterm or LBW neonates.<sup>165</sup>

The presence of intact neutrophil phagocytic function is a critical factor involved in controlling replication and spread of staphylococci.<sup>166</sup> The bone marrow's ability to respond to infection with rapidly enhanced production and maturation of neutrophil precursors is limited compared with adults.<sup>167</sup> Neutrophils from newborns have relatively diminished motility toward chemoattractants compared with that in cells from older children and adults,<sup>168</sup> in part the result of diminished chemotactic factors, such as the complement-derived C5a and the CXC chemokine interleukin-8 (IL-8).<sup>169,170</sup> Neutrophils from young infants also exhibit decreased diapedesis across endothelium, possibly because of impaired capacity to upregulate endothelial cell expression of the CR3 receptor.<sup>171</sup> Beyond decreases in neutrophil number, chemotaxis, and transepithelial migration, the capacity for neutrophil adherence and phagocytosis is



reduced in neonates, largely because of deficiencies in opsonins, including complement and specific antibody.<sup>166</sup> Compared with adult cells, neonatal neutrophils are less able to produce DNA-based extracellular traps (NETs) important for capturing and killing extracellular bacteria.<sup>172</sup>

Phagocytic killing appears to be intact in normal-term newborns but may be compromised in stressed infants, at least in part because of reduced production of reactive oxygen species.<sup>173,174</sup> A defective oxidative burst of neutrophils from preterm neonates on exposure to CoNS has been documented.<sup>175,176</sup> The multifaceted antioxidant capacities of *S. aureus*, including catalase and the carotenoid staphyloxanthin pigment likely underpin its prominent role as an opportunistic pathogen in such settings, as well as in patients with marginal patients with chronic granulomatous disease (CGD), where defects in reduced nicotinamide adenine dinucleotide phosphate oxidase lead to marginal oxidative burst function. CGD may occasionally present with *S. aureus*, *Serratia* spp., or *Aspergillus* spp. in the neonatal period.<sup>177</sup>

Specific antibody is less important than complement in opsonization of *S. aureus* and plays a limited role in defense against neonatal staphylococcal disease.<sup>178</sup> For example, in general, there is no correlation between antibody titers against *S. aureus* and the likelihood of asymptomatic carriage versus clinical disease.<sup>179,180</sup> Consistent with this information, an attempt many years ago to protect the newborn from staphylococcal disease by immunizing the mother near term was unsuccessful.<sup>181</sup>

CoNS-induced cytokine production from human neonatal monocytes varies with gestational age,<sup>182,183</sup> and preterm newborn monocytes show reduced IL-1 $\beta$ , IL-6, IL-8, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production despite adult-level expression of Toll-like receptor 2.<sup>184</sup> The block in TNF- $\alpha$  production may contribute to impaired antistaphylococcal neutrophil responses because TNF- $\alpha$  plays an important role in neutrophil activation.<sup>165</sup>

In most cases of neonatal staphylococcal disease, the role of T cells is unclear. In animal models, T cells are found to contribute to the development of abscesses during *S. aureus* infection.<sup>185</sup> T cells are centrally involved, however, in the immune response to several *S. aureus* toxins, including toxic shock syndrome toxin-1 (TSST-1), the staphylococcal enterotoxins, and the staphylococcal exfoliative toxins (ETA, ETB, ETC, and ETD), and thus in associated pathogenesis. The consequence of this dysregulated T-cell overactivation is proliferation of a large proportion of T cells and release of a number of cytokines, including TNF- $\alpha$ , IL-1, and interferon- $\gamma$ .<sup>186</sup> These molecules are major contributors to the systemic manifestations of staphylococcal scalded skin syndrome (SSSS), TSS, and food poisoning.

## Pathology

The most characteristic pathologic lesion associated with *S. aureus* infection is a local abscess, consisting of necrotic tissue, fibrin, and a large number of live and dead neutrophils. Similarly, CoNS infection is characterized by infiltration of neutrophils, usually with moderate necrosis. Other pathologic findings are described next in the sections on clinical manifestations.

## Clinical Manifestations

Staphylococci are capable of producing a profound variety of clinical syndromes in the newborn infant, including those with high mortality rates, as was reviewed more than 50 years ago.<sup>187</sup> The clinical manifestations of staphylococcal infection are most prominently a function of two factors: the gestational age of the infants, with extremely LBW infants at highest risk of infection and subsequent complications, and the strain of staphylococcus causing the infection, with the CoNS generally causing more mild infection compared with *S. aureus*, particularly relevant to recent CA-MRSA. As noted in earlier sections of this chapter, staphylococci are armed with an impressive array of virulence factors. They may merely colonize skin, respiratory or gastrointestinal tract mucosa without apparent harm to the host, or cause invasive, lethal disease. CoNS are most often benign colonizers of skin and gastrointestinal tract in the newborn, causing frequent but relatively mild infections in the hospitalized premature infant. In contrast, coagulase-positive strains (*S. aureus*) are more commonly associated with clinically aggressive, invasive infections. The subsequent sections provide both a general overview of clinical manifestations as well as organ-specific manifestations.

### BACTEREMIA/SEPSIS

The most common manifestations of invasive staphylococcal infection are bacteremia/sepsis. Studies describing symptomatic bacteremia in neonates, both community-acquired and hospital-acquired, provide an overall framework in which CoNS and *S. aureus* infection can be defined and include both early-onset sepsis (EOS) and LOS syndromes.<sup>7,23,26,188-201</sup>

EOS is most often related to acute infectious complications of late pregnancy and delivery, or colonization of the infant at birth and subsequent development of clinical symptoms within the first 48 to 72 hours of life. The signs and symptoms associated with staphylococcal septicemia usually are nonspecific and include disturbances of temperature regulation, respiration, circulation, gastrointestinal function, and central nervous system (CNS) activity. Hypothermia is more common than fever and often is observed as the initial sign. Respiratory distress frequently manifests as episodes of apnea and bradycardia, particularly in infants who weigh less than 1500g. Other abnormalities related to respiration include tachypnea, retractions, and cyanosis. In 20% to 30% of infants, gastrointestinal abnormalities develop, including poor feeding, regurgitation, abdominal distention, diarrhea, and bloody stools. Evidence of poor perfusion can include mottling, poor capillary refill, and metabolic acidosis. In some infants, lethargy, irritability, or poor suck may also be noted.

The incidence of EOS caused by *S. aureus* appears to reflect the characteristics of circulating strains and varies by year and region of the world (see “Epidemiology and Transmission”). Clinical descriptions of staphylococcal sepsis include a positive blood culture, usually in the context of nonspecific clinical signs and symptoms that may include apnea, bradycardia, irritability, poor feeding, abdominal distention, lethargy, hypotonia, hypothermia or hyperthermia, hypotension with poor tissue perfusion, and cyanosis and

increased oxygen requirement with respiratory distress. In a 75-year collection of data from Yale-New Haven Hospital,<sup>6</sup> *S. aureus* represented the etiology of EOS from 28% (1928-1932) to 3% (1979-1988), with a rate in the present era (1989-2003) of 7%. In the Yale experience, mortality from all causes of EOS dropped from about 90% with the earliest data set to approximately 5% of all inborn infants from 1989 to 2003. In NICHD Neonatal Research Network data from 2006 to 2008, approximately 3.7% of initial episodes of LOS or meningitis among VLBW infants (<1500 g) were caused by *S. aureus*, with roughly one third of cases being MRSA<sup>23</sup>; similar findings were reported in a study based at Columbia University Medical Center.<sup>24</sup>

EOS caused by CoNS is reported extremely infrequently, likely because of the noninvasive nature of most strains. These reports may reflect true infection, particularly in VLBW infants,<sup>193,202</sup> although the extent of symptoms attributable to infection in these infants is difficult to assess. Otherwise, particularly for term infants, a positive culture of blood for CoNS may represent a contaminant unrelated to the underlying illness. True CoNS infection is very uncommon in infants with birth weight greater than 2000 g and gestation longer than 34 weeks.<sup>203</sup>

When considering neonatal LOS syndrome, occurring after the fifth day of life in hospitalized infants, both *S. aureus* and CoNS are well-documented pathogens. In the NICHD Neonatal Research Network, *S. aureus* was the second most common pathogen to cause LOS in VLBW (401-1500 g) infants.<sup>5</sup> CA-MRSA produces particularly devastating infection, with 7 of 8 infants hospitalized in the NICU of Texas Children's Hospital in Houston presenting in septic shock; the case-fatality ratio was 38% in this series, despite appropriate support and antimicrobial therapy.<sup>204</sup> In a maternity hospital in Houston during the same period, mortality attributable to the invasive *S. aureus* infection was 6%, with infection-attributable late sequelae of 12%.<sup>204</sup> In this report, only 3 of 39 *S. aureus* infections were caused by MRSA; all 3 infants recovered without sequelae. In a retrospective review of 12 neonates with bacteremia caused by MSSA, compared with 11 neonates infected by CA-MRSA, collected from 1993 to 2003 in Tel Aviv, mortality rates were virtually identical: 25% versus 27%, respectively.<sup>198</sup> In a larger series of 90 infants from Taiwan with bacteremia caused by MRSA, 75% of infants were premature, 54% of infections were thought to be catheter related, 21% were associated with skin and soft tissue infections, 17% associated with pneumonia, 8% with bone and joint infection, 3% with meningitis, and 3% with peritonitis.<sup>205</sup> This rate of metastatic infection attributed to MRSA appears greater than that noted with MSSA and is clearly greater than rates seen with CoNS bacteremia. Of those infants with resolved MRSA infection, 10% had at least one recurrence. At Duke University Medical Center, mortality and neurodevelopmental outcomes in infants with bacteremia caused by MSSA (median age, 26.5 days) were compared with MRSA (median age, 26 days).<sup>206</sup> Although the duration of staphylococcal bacteremia was shorter in neonates with MSSA (1 vs. 4.5 days), the mortality and neurodevelopmental outcomes were statistically similar to infants infected with MRSA. Similar clinical outcomes were reported comparing MSSA and MRSA infection in the NICHD Neonatal Research Network data from 2006 to 2008.

The largest burden of disease in LOS caused by staphylococci is catheter-related CoNS bacteremia in premature infants. In the NICU, CoNS cause between 40% and 60% of all bacteremic episodes.<sup>5,7,207-209</sup> Rates of catheter-associated bacteremia have been tracked by the U.S. Centers for Disease Control and Prevention (CDC),<sup>210</sup> and other collaborative groups, including the Pediatric Prevention Network<sup>188</sup> and the Vermont Oxford National Evidence-Based Quality Improvement Collaborative for Neonatology.<sup>211</sup> Clinical manifestations of infection are frequently related to the gestational and chronologic age of the newborn but are most often nonspecific. In a retrospective review of invasive staphylococcal infections in a maternity hospital in Houston from 2000 to 2002, bacteremia was present in 94% of 108 infants with invasive CoNS infection, resulting in a wide range of nonspecific symptoms, including apnea and bradycardia in 52%, an increased oxygen requirement in 90%, lethargy in 31%, abdominal distention in 30%, increased blood pressure support requirement in 22%, and temperature instability in 18%.<sup>202</sup> Similar findings were published by investigators in the NICHD Neonatal Research Network,<sup>23</sup> highlighting the burden of disease in the VLBW infants.

CoNS infections have often been associated with many risk factors (see "Epidemiology and Transmission"), but the ultimate outcome of infants infected with CoNS may more closely follow their comorbidities than be linked to bacterial pathogenicity. In a review of data collected by the Pediatrix Medical Group (Winchester, VA), Benjamin and colleagues<sup>191</sup> noted that the survival of LBW infants ( $\leq 1250$  g) after a positive blood culture for CoNS was virtually identical (8%) to that of infants evaluated for sepsis yielding sterile blood cultures, in contrast to much higher rates of mortality with gram-negative organisms or *Candida*.<sup>191</sup> Similar findings suggesting lack of attributable mortality to CoNS bacteremia documented that, for infants who ultimately died of any cause, death occurred more than 7 days after the positive blood culture for CoNS in 75% of infants.<sup>5</sup> These findings were also confirmed in VLBW infants in Israel when reporting on 3462 episodes of LOS, documenting a mortality within 72 hours of CoNS bacteremia of only 1.8%.<sup>212</sup> Others have suggested that persisting positive blood cultures for CoNS, despite appropriate antibiotic coverage, are associated with an increase in overall complications, with a mortality as high as 7%.<sup>190</sup>

Treatment of catheter-associated CoNS infections remains controversial. Karłowicz and colleagues<sup>213</sup> prospectively evaluated treatment with vancomycin versus catheter removal. In those treated with vancomycin who experienced clearing of bacteremia within 1 to 2 days, success without catheter removal occurred in 79%, whereas in those with persisting bacteremia of 3 to 4 days, the success rate declined to 44%, and in those with bacteremia persisting beyond 4 days, none were successfully treated with medical therapy alone, a finding similar to that by Benjamin and colleagues<sup>214</sup> in a retrospective review, in which the rate of metastatic infection increased significantly after four or more positive cultures. Others have attempted to limit the empirical use of vancomycin in the NICU by comparing outcomes using vancomycin-containing empirical regimens during one period of study, with cloxacillin-containing regimens during another. When all-cause mortality was assessed at 14 days after positive blood culture,

0 of 45 infants receiving vancomycin versus 4 of 37 infants not receiving vancomycin had died. When examined on an individual case basis, only one of the deaths was possibly attributed to CoNS sepsis.<sup>215</sup>

## TOXIC SHOCK SYNDROMES

In addition to clinical manifestations related to bacteremia, toxin-mediated clinical disease may also occur, including SSSS (see later), TSS,<sup>216</sup> and neonatal TSS-like exanthematous disease.<sup>217</sup> TSS is caused by pyrogenic toxin superantigens produced by *S. aureus*. These superantigens include TSST-1 and several enterotoxins, most commonly staphylococcal enterotoxin serotype B or C.<sup>218,219</sup> TSS has been described in a 4-day-old term infant male, with poor feeding and vomiting at 3 days of age, followed by hypotension, respiratory distress, and multiorgan failure on day 4 of life. Generalized erythema developed at 6 days of age. This infant was colonized on the umbilicus with a methicillin-susceptible strain that produced staphylococcal enterotoxins C, G, and I.<sup>216</sup>

A similar disease caused by MRSA has been described in Japan, producing erythema in association with either thrombocytopenia, elevated C-reactive protein (CRP), or fever<sup>217</sup>; this presentation has been termed neonatal toxic shock syndrome–like exanthematous disease (NTED) (Fig. 14-5).<sup>217</sup> Since the time of the first description, surveys in Japan have shown that up to 70% of Japanese hospitals have reported a similar illness in neonates.<sup>220</sup> The causative strains all carried the TSST-1 gene, as well as the staphylococcal enterotoxin C gene.<sup>221</sup> The pathophysiology of NTED begins with colonization with MRSA, a common occurrence among Japanese newborns. Typically, the colonizing strain of MRSA produces TSST-1,<sup>221</sup> and the symptoms of the disease are related to the overactivation of TSST-1–reactive T cells.<sup>222</sup> NTED does not develop in all infants who are colonized with TSST-1–producing MRSA, suggesting that protection from this illness may be mediated by the transplacental transfer of maternal antibody directed against TSST-1.<sup>217</sup>



**Figure 14-5** Typical exanthem in a full-term infant with neonatal toxic shock syndrome–like exanthematous disease. (From Takahashi N, Nishida H, Kato H, et al: Exanthematous disease induced by toxic shock syndrome toxin 1 in the early neonatal period, *Lancet* 351:1614–1619, 1998.)

## ENDOCARDITIS

Although endocarditis in the neonate is a rare entity, autopsy studies from the 1970s revealed unsuspected endocarditis in 0.2% to 3% of neonates who came to autopsy.<sup>223,224</sup> Historically, *S. aureus* has been the predominant bacterial pathogen among neonates with endocarditis,<sup>225</sup> but more recent reports indicate that CoNS is now most common.<sup>226-229</sup> Premature infants with prolonged central catheter bacteremia and infants with congenital heart disease are most likely to develop *S. aureus* endocarditis in association with bacteremia.<sup>227,229-233</sup> Endocarditis has also been described in infants infected by CA-MRSA.<sup>234</sup>

The signs and symptoms of endocarditis in neonates often are nonspecific and similar to those of other conditions such as sepsis or congenital heart disease, including poor feeding, tachycardia, and respiratory distress.<sup>225</sup> Clinical features, in general, may not be able to distinguish bacteremia with endocarditis from infants with bacteremia without endocarditis.<sup>229</sup> Murmurs can be appreciated in up to 75% of neonates with endocarditis, with hepatosplenomegaly present in up to 50%, skin abscesses in 44%, arthritis in 12%, and petechiae in 12%. Blood cultures and echocardiography are the most important diagnostic tests, although urine cultures may be positive in up to 38%.<sup>227,232</sup> The yield of a single blood culture has been reported to be between 77% and 97%. When three blood cultures are obtained, the yield approaches 100%.<sup>232</sup> When persistence of CoNS bacteremia occurs in VLBW infants, it is critical to perform echocardiography such that endocarditis be excluded.<sup>235</sup>

All neonates with *S. aureus* bacteremia should be evaluated by echocardiogram. The thin chest wall of the neonates makes echocardiography a highly sensitive tool for diagnosis of endocarditis in this age group. The limitations of this technique include the inability to detect lesions less than 2mm in diameter and to differentiate between vegetations and other masses, such as thrombi.<sup>227</sup> In all age groups of children, up to 12% of those bacteremic with *S. aureus* may have clear evidence of endocarditis; children with underlying congenital heart disease and *S. aureus* bacteremia demonstrate a much greater risk of endocarditis compared with those with no cardiac malformations (53% vs. 3%).<sup>230</sup> Mortality in children with *S. aureus* bacteremia and endocarditis has been reported as high as 40%.<sup>230</sup>

In the Australasian Study Group for Neonatal Infections, bacteremia caused by CoNS in 1281 infants during 1991 to 2000 was associated with endocarditis in 3 (0.2%); in those with bacteremia caused by *S. aureus*, endocarditis occurred in 3 of 223 (1.3%) infants infected with methicillin-susceptible strains and in 1 of 65 (2%) infants infected with CA-MRSA strains. Historically, the prognosis for neonates with endocarditis has been grave. Series published in recent years report disease-specific survival rates ranging from 40% to 70%.<sup>227,229,232</sup>

## PUSTULOSIS, CUTANEOUS ABSCESS, CELLULITIS

For infants presenting to Texas Children's Hospital in Houston, skin infection was the most common manifestation of staphylococcal disease (88%) in term or late preterm infants ( $\geq 36$  weeks gestation). Of those with skin infection, about two thirds presented with cellulitis or abscess,

whereas one third presented with pustulosis, a localized, nonsystemic, invasive cutaneous form of infection. Of interest, two thirds of all *S. aureus* infections were caused by CA-MRSA, with both CA-MRSA and MSSA presenting with skin or invasive infection in roughly equal percentages; the proportion of infections caused by CA-MRSA increased over the period of observation, from 2001 to 2006.<sup>236,237</sup> A similar experience was reported from Chicago, in which 11 infants younger than 1 month were culture positive for CA-MRSA, with cutaneous lesions consisting of pustules and vesicles, most commonly present in the diaper area. Resolution of cutaneous infection occurred with the use of mupirocin ointment. No infant required surgical drainage, and no infant developed systemic manifestations of disease or required hospitalization with intravenous antibiotic therapy.<sup>238</sup> Similar clusters of skin-only pustules and vesicles have been reported from other centers.<sup>239</sup>

Evaluation of newborn infants discharged from the hospital, but readmitted within 30 days of age, provided a somewhat different profile of clinical disease caused by staphylococci.<sup>236</sup> Infants infected with MRSA presented at 7 to 12 days of age, in contrast to those infected with MSSA, whose presentations occurred evenly spaced over the first month of life. The majority of these infants (87% for MRSA and 86% for MSSA) presented with skin and soft tissue infection. Cellulitis with or without abscess was responsible for about two thirds of hospitalizations. Pustulosis,<sup>240</sup> primarily involving skin covered by a diaper, was the most prominent sign in approximately one third of infections. Invasive disease occurred in about 10% of infants, including bacteremia, urinary tract infection, osteomyelitis, myositis, and empyema.

A study of the clinical characteristics of neonates hospitalized in a level 3 NICU (40 beds) and cultured weekly from the nose and inguinal areas, to assess ongoing colonization status, demonstrated that of 152 infants known to be colonized over the study period of 2002 to 2004, 6 (3.9%) developed MRSA sepsis, 3 (2.0%) developed conjunctivitis, 2 (1.3%) developed chest tube–site wound infections, and 2 (1.3%) developed cellulitis.<sup>241</sup>

## ADENITIS AND PAROTITIS

In the newborn, *S. aureus* cervical adenitis can be another manifestation of nursery colonization. At least two outbreaks of cervical adenitis resulting from nurseries were reported in 1972. One outbreak involving 25 infants had an attack rate of 1.9%, and another involving 9 infants had an attack rate of 5.6%.<sup>242,243</sup> As with other manifestations of nursery-associated *S. aureus* disease, illness usually appears after discharge from the hospital. The mean incubation periods in the two outbreaks in England were 86 and 72 days, respectively. Because of the delay in onset of disease, confirmation of a nursery as the source of the infection may be difficult and would necessitate careful epidemiologic investigation. Deep neck abscesses have been reported secondary to MSSA or MRSA.<sup>244,245</sup> Neonatal suppurative parotitis is an uncommon infection among newborns, occurring with an incidence of 13.8 per 10,000 admissions.<sup>246,247</sup> Premature neonates (one third of cases) and males appear to be at highest risk for suppurative parotitis, which is most frequently caused by *S. aureus*.<sup>248,249</sup> Fever was seen in less than half of them (47%). Premature babies constituted a third of the patients. Diagnosis of suppurative parotitis relies on the clinical findings of parotid swelling, fever, and pus expressed from Stensen's duct on compression of the parotid gland.<sup>247,250</sup>

## BREAST INFECTION

A series of 39 neonatal breast abscesses caused by *S. aureus* were reported by Rudoy and Nelson<sup>251</sup> from Dallas in 1975. These infants developed infection most commonly during the second week of life, when neonatal breast tissue is still enlarged in response to transplacental estrogens. The infection is clinically easy to detect, with acute onset of swelling, erythema, and tenderness of the affected breast, with progression of the infection over several hours, occasionally spreading to surrounding tissues (Fig 14-6). Spontaneous drainage of purulent material from the infant's breast



**Figure 14-6 A and B,** Left breast abscess in a 12-day-old infant. The abscess extends toward the right side of chest and up over the arm. The infant responded well to incision and drainage and antibiotic treatment.

may or may not occur. Culture and Gram stain of purulent discharge is diagnostic. Management includes systemic antistaphylococcal antimicrobials as well as careful surgical drainage of abscessed tissue within the breast, particularly in female infants. In the report from Dallas, one third of infant girls followed into early adolescence were documented to have decreased breast size as a complication of the infection.<sup>251</sup>

In other series of cases in which follow-up histories were obtained, a decrease in breast size was noted in two of six individuals who were examined at the ages of 8 and 15 years, respectively.<sup>251,252</sup> A series of three female neonates with necrotizing fasciitis as a complication of breast infection/abscess were collected from the Hôpital Necker in Paris over a 30-year period, all caused by MSSA, with no infant having a concurrently positive blood culture. All infants survived after extensive surgery and prolonged antibiotic therapy. In one of three cases followed through puberty, breast development did not occur on the affected side.<sup>253</sup> Antimicrobial therapy should be provided intravenously until a clear and substantial response can be documented. In locations with a high prevalence of CA-MRSA, therapy should include clindamycin or vancomycin.

### FUNISITIS, OMPHALITIS, NECROTIZING FASCIITIS

Funisitis, mild inflammation of the umbilical stump with minimal drainage and minimal erythema in the surrounding tissue, is a local, noninvasive entity. However, infections of the umbilical stump may become invasive, and occur in a full spectrum of clinical presentations from funisitis, to massive abdominal wall inflammation with erythema and indurative edema associated with necrotizing fasciitis. In an attempt to define the stages of the spectrum of infection, some experts have separated the infection into distinct categories: category 1, funisitis/umbilical discharge (shaggy unhealthy umbilical stump, malodorous, and/or purulent discharge); category 2, omphalitis with abdominal wall cellulitis (periumbilical erythema, superficial tenderness besides findings in category 1); category 3, omphalitis with systemic sepsis; category 4, omphalitis with fasciitis (umbilical necrosis with extensive local disease, periumbilical ecchymosis, crepitus bullae, and evidence of involvement of superficial and deep fascia).<sup>254</sup>

Cultures of umbilical tissue in all categories of infection often yield several organisms, including *S. aureus* and CA-MRSA.<sup>237,255</sup> Management of categories 1 to 3 is usually with aggressive local care and systemic, broad-spectrum antibiotic therapy active against enteric bacilli, anaerobes, and *S. aureus*, with an option to provide oral therapy only for infants in category 1, if close observation and frequent reexamination can be arranged.

The most life-threatening entity, necrotizing fasciitis, requires immediate administration of broad-spectrum antibiotics and supportive care, with aggressive surgical débridement. As *S. aureus* may be just one of several pathogens cultured, the exact role of *S. aureus* in the overall clinical disease process cannot be accurately assessed. Of 7 infants presenting at 4 to 14 days of age with necrotizing fasciitis in Los Angeles, 4 were culture-positive for *S. aureus* in a mixed infection.<sup>256</sup> In Muscat, Oman, 10 of 14 neonates

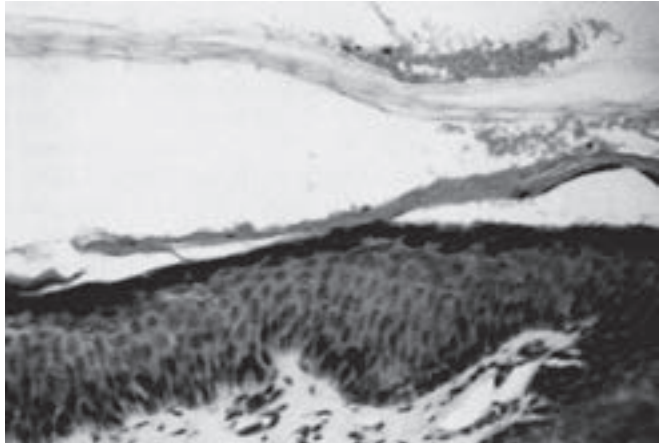
had *S. aureus* cultured from umbilical tissue, including 1 infant positive for MRSA, with 3 of these 10 infants having concurrent staphylococcal bacteremia.<sup>257</sup> Despite aggressive management, the mortality rates of polymicrobial necrotizing fasciitis have been 60% to 70% from sites in the United States,<sup>256,257</sup> suggesting that earlier recognition with aggressive surgical management and critical care support, antimicrobial therapy that includes activity against *S. aureus*, or CA-MRSA if appropriate, may be necessary to improve outcomes. A report of three cases of MRSA necrotizing fasciitis from the Chang Gung Children's Hospital in Taiwan was published in 1999.<sup>258</sup> Mastitis was a nidus for extension to necrotizing fasciitis in another series of neonatal patients.<sup>253</sup> Single cases of neonates from San Diego and Chicago were also reported.<sup>259,260</sup>

Neonates present for medical attention between 5 and 16 days of age with acute development of symptoms over 24 to 48 hours and rapid spread of erythema with indurative edema of infected tissues that have not been known to be previously traumatized. The infants may appear systemically ill with fever, irritability, and a laboratory evaluation suggesting acute inflammation with an elevated peripheral white blood count, CRP, and frequently a blood culture that is positive for *S. aureus*. Although imaging should not delay emergent surgical débridement, magnetic resonance imaging (MRI) is the preferred modality in adults and presumably infants, to define the characteristic soft tissue characteristics of necrotizing fasciitis.<sup>261,262</sup> In addition to broad-spectrum antimicrobials outlined above and surgical débridement, the role of hyperbaric oxygen treatment is poorly defined, with no prospective, randomized clinical trial data, and only single cases or small case series that may or may not support adjunctive hyperbaric oxygen therapy.<sup>258,262,263</sup>

### STAPHYLOCOCCAL SCALDED SKIN SYNDROME AND BULLOUS IMPETIGO

Staphylococcal scalded skin syndrome has been reported in both full-term and premature infants,<sup>264-268</sup> with the first reported series of patients in 1878 from Prague by Ritter von Rittershain of a clinical infection that is likely to have included patients with SSSS.<sup>269</sup> Clinical characteristics in neonates are similar to those in infants and older children<sup>270</sup> with acute onset of infection associated with erythema, either macular or generalized, usually starting on the face and moving to the trunk within 24 hours. Erythema is accentuated in the flexor creases of the extremities, similar to streptococcal toxin disease but with minimal mucus membrane erythema. Within 48 hours, the involved tender skin, primarily on the face, diaper area, and extremities, begins to form superficial, clear, flaccid bullae that subsequently break, revealing bright red, moist skin. These lesions demonstrate a separation of tissue layers within the epidermis, at the junction of the stratum spinosum and stratum granulosum, because of the effect of staphylococcal exfoliative toxins A and B on desmoglein-1 (see "Pathogenesis of Disease"). The characteristic histologic feature of SSSS is intraepidermal cleavage through the granular layer, without evidence of epidermal necrosis or inflammatory cell infiltrate (Fig. 14-7).<sup>271</sup> This appearance is distinct from that in

toxic epidermal necrolysis, characterized by a subepidermal split- and full-thickness necrosis of the epidermis. Desquamation may be local, under the bullae, or generalized (Fig. 14-8). Before formation of bullae, erythematous skin will demonstrate intraepidermal separation when gentle tangential pressure is applied (Nikolsky sign), resulting in blister formation. These cutaneous findings may occur in the context of low-grade fever in about 20% of infants. Given the relatively high layer of epidermis involved, no major clinical sequelae occur as there are no substantial



**Figure 14-7** Photomicrograph of a skin biopsy from a patient with staphylococcal scalded skin syndrome, stained with hematoxylin and eosin. The histologic appearance is characterized by epidermal splitting at the granular layer of the epidermis. Magnification is approximately  $\times 200$ . (From Hardwick N, Parry CM, Sharpe GR: Staphylococcal scalded skin syndrome in an adult. Influence of immune and renal factors, *Br J Dermatol* 132:468–471, 1995.)

fluid, electrolyte, or protein losses, in contrast to erythema multiforme involving the dermal-epidermal junction. After appropriate antimicrobial therapy, the denuded skin dries within the subsequent few days, and in the absence of superinfection, heals completely within a few weeks with no scar formation.

In a recent 8-year (2004-2012) retrospective review of 39 neonates diagnosed with SSSS, mean patient age was 17 days, boys were more commonly affected (64%), the face was the most common body part affected and the area most frequently initially affected, and pneumonia the most common complication (three fourths of cases).<sup>272</sup> Fever, leukocytosis, or elevated CRP levels were uncommon; the median length of hospitalization was 9 days; and all neonates were cured without scarring after antibiotic treatment.<sup>272</sup>

Localized staphylococcal infection complicated by SSSS may also occur with the presence of wound infections, cutaneous abscesses, or conjunctivitis.<sup>266,268,273</sup> Bacteremia is very uncommon with SSSS but has been reported.<sup>274,275</sup> Although infection is most commonly described in full-term neonates during the first few months of life, infections in premature infants, including those with extremely low birth weight, have also been described.<sup>264-267</sup> Scarletina, as the only clinical manifestation of infection caused by an epidemic strain of SSSS-causing *S. aureus*, has also been observed.<sup>268</sup>

Congenital SSSS infection, acquired before delivery as a function of maternal amnionitis, has been reported in both term<sup>275,276</sup> and preterm<sup>277</sup> infants, with a mortality rate that may be higher than the extremely low rate documented for disease acquired postnatally. Outbreaks of disease among hospitalized infants in nurseries have occurred, but most have been effectively stopped with standard infection-control practices.<sup>268,278</sup>



**Figure 14-8** Generalized staphylococcal scalded skin syndrome in a previously well newborn infant. **A** and **B**, The characteristic well-demarcated erythematous superficial exfoliation, with areas of skin sparing, can be seen. (From Ladhani S, Joannou CL, Lochrie DP, et al: Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome, *Clin Microbiol Rev* 12:224–242, 1999.)

## PNEUMONIA

Neonatal pneumonia caused by *S. aureus* has been described for decades, often reported to occur in community epidemics in infants during the first month of life. These infections, even before the advent of CA-MRSA, have been known to cause severe disease with a high mortality rate that may reflect virulence of strains circulating at that time.<sup>187,279,280</sup>

A lower respiratory tract infection may occur as a primary pneumonia as the sole clinical manifestation of infection caused by *S. aureus*, with acquisition of the organism after contact with family members or hospital staff. Pneumonia may also occur as part of more generalized, invasive, disseminated staphylococcal infection. In a recently reported large series of neonatal sepsis/bacteremia cases, pneumonia caused by either CoNS or *S. aureus* is only rarely listed as a primary diagnosis, or a complication, without details provided about the clinical presentation of lower respiratory tract disease.<sup>5,188,189,201,209</sup> The infection is often hospital acquired in a neonate with underlying lung disease, most commonly chronic lung disease (bronchopulmonary dysplasia), especially in those receiving concurrent mechanical ventilation.

An early study of neonatal staphylococcal pneumonia was reported from New Zealand in 1956 during an epidemic of primarily cutaneous infections. Eight infants died of pneumonia in this epidemic, and they presented at 2 to 3 weeks of age with irritability and poor feeding, followed by dyspnea, cough, and fever.<sup>279</sup> Death occurred in these infants between 1 and 5 days after admission, with autopsy findings documenting empyema, consolidation, and abscess formation. In a study of community-acquired *S. aureus* infection in neonates from Houston between 2001 and 2005, infants were described who had no underlying disease, no indwelling catheters, and no previous hospitalization. Of 89 neonates identified with *S. aureus* infection, only 1 had a primary lung infection, caused by CA-MRSA, producing a necrotizing pneumonia complicated by pneumothorax and empyema, requiring video-assisted thoracoscopic surgery and chest tube drainage. Other cases of severe CA-MRSA neonatal pneumonia have been reported, including hospital-acquired infections in premature neonates.<sup>26,194,281</sup> In hospitalized, bacteremic neonates with CA-MRSA in Houston, 2 of 8 had lung involvement: a 24-week gestation 14-day-old infant with empyema, pneumatoceles, and concurrent endocarditis, and a 28-week gestation infant had multiple comorbidities, including bronchopulmonary dysplasia with infection acquired at 411 days of age, characterized by lung abscess. Both infants died. Two fatal cases of MRSA pneumonia complicated by pleural empyema in neonates from Quebec were also reported.<sup>282</sup>

In a point-prevalence survey of neonatal infections in 29 Pediatric Prevention Network NICUs in the United States and Canada, 116 NICU-acquired infections were reported, with 15 (13%) respiratory associated, virtually all in infants with birth weights of 501 to 1500 g, with only 2 infections associated with CoNS, and only 1 associated with *S. aureus*.<sup>188</sup> In a review of invasive staphylococcal infections of hospitalized neonates admitted to level 2 or level 3 nurseries in Houston, 3 of 41 infants with *S. aureus* infection and 14 of 108 with CoNS infection were documented to have pneumonia.<sup>202</sup> In this population of infants, many had comorbidities, including

respiratory distress syndrome in 85% of neonates ultimately diagnosed with any invasive *S. aureus* infection and in 95% of those with CoNS. Similarly, bronchopulmonary dysplasia was documented in 65% of *S. aureus* infected infants and 75% of those infected by CoNS. The Australasian Study Group for Neonatal Infections collected data on infants with documented bacteremia, occurring at between 48 hours and 30 days of age. Of 1281 episodes of CoNS bacteremia, only 6 (0.5%) were documented to have pneumonia,<sup>209</sup> in contrast to 223 with MSSA bacteremia associated with 9 (4%) cases of pneumonia, and 65 cases of MRSA bacteremia associated with 8 (12%) cases of pneumonia.<sup>189</sup>

## MENINGITIS

Meningitis is infrequently encountered in neonates with *S. aureus* bacteremia<sup>202,236,237</sup> but may be found when a lumbar puncture is performed after empirical antibiotic therapy has been started. In a large series of 90 episodes of MRSA bacteremia in neonates in a Taiwanese NICU, 2 infants were noted to have meningitis.<sup>205</sup> In a report from Australia and New Zealand, meningitis was reported in 5 of 223 infants with MSSA bacteremia (2.2%), and 3 of 65 infants had MRSA bacteremia (5%).<sup>209</sup> However, in most reports where cerebrospinal fluid (CSF) white blood cell counts are provided, they are often below 200 cells/mm<sup>3</sup>, suggesting that these infants with a CSF pleocytosis and negative CSF cultures may not have true staphylococcal bacterial meningitis. Virtually no infant from any series had a positive CSF culture for *S. aureus*, including 12 bacteremic infants with a documented pleocytosis from Texas Children's Hospital.<sup>237</sup> It is possible that the pleocytosis represents the entry of staphylococcal cell wall components or inflammatory mediators into CSF during bacteremia, perhaps facilitated by capillary leak that often accompanies staphylococcal sepsis. Other possibilities include very early bacterial meningitis or a staphylococcal parameningeal focus of infection that was not identified in infants nor included in the reports.

Among 1281 episodes of CoNS sepsis, 5 (0.4%) were reported to be associated with meningitis.<sup>209</sup> An additional 2 cases of CoNS meningitis were reported in premature infants from a single institution, born at 24 and 25 weeks of gestation, 1 with a grade IV intraventricular hemorrhage on the fifth day of life, developing symptomatic disease at 20 days of age the second premature infant did not have an intraventricular hemorrhage, but developed disease at 18 days of age. Multiple cultures in both infants confirmed infection, caused by *S. epidermidis* in 1 infant, and *S. capitis/S. warneri* in the other.<sup>283</sup> A major risk factor for CoNS meningitis is prior neurosurgery, in particular ventriculoperitoneal shunt insertion.<sup>284</sup>

## BRAIN ABSCESS

Brain abscesses caused by *S. aureus* have been described in neonates, most often as a complication of sepsis.<sup>285-287</sup> Clinical presentation includes nonspecific symptoms of systemic infection, a bulging fontanel, and may not include focal neurologic deficits. The key to diagnosis includes ultrasonography or computed tomography (CT) imaging of the head, with the administration of an intravenous contrast agent, and if not contraindicated, an evaluation of lumbar CSF.

Surgical drainage of the abscess is usually performed after prolonged antistaphylococcal therapy. Long-term outcome has included neurologic deficits of varying degrees. A case of multiple brain abscesses caused by complicating MRSA bacteremia in a 29-week-gestation premature infant was successfully treated and recently reported.<sup>288</sup>

Neonates with intraventricular hemorrhage, acute hydrocephalus, congenital malformation, or CNS parenchymal injury, will often require management of increased intracranial pressure by repeated lumbar puncture, or by placement of a shunt originating in the CSF ventricles.<sup>289,290</sup> CoNS clearly represent the most common organisms to infect shunt material, producing mild-to-moderate inflammation and systemic signs and symptoms of infection<sup>289-293</sup>; occasional infection caused by *S. aureus* has also been reported.<sup>290,293</sup> Shunt removal is the preferred method of treatment because sterilization of in situ shunt material is often quite difficult.<sup>291</sup> Treatment with high-dose systemic antimicrobials active against the isolated pathogens, usually vancomycin, from 3 to 10 days post-shunt removal is recommended, depending on the clinical status and the microbiologic response to treatment. Some authors also recommend using intraventricular vancomycin therapy if therapeutic ventricular CSF concentrations cannot be achieved with systemic therapy.<sup>291,294,295</sup> The role of linezolid in the treatment of shunt infections remains to be defined but may represent an option for CA-MRSA strains that are not fully susceptible to vancomycin.<sup>296</sup> Although the CoNS infections are seldom associated with acute toxicity or mortality, management with long-term antibiotic therapy and repeated surgical interventions for removal and placement of shunts each carry risks to the neonate.

## OSTEOMYELITIS AND SEPTIC ARTHRITIS

Bone and joint infections have long been known to occur with invasive staphylococcal infection in the neonate, with rates of late sequelae as high as 50%.<sup>297-302</sup> In contrast to infections in older children, the usual distinction between infection of the bone and infection of the joint in the neonate is not as easily made given the unique neonatal anatomy, with easy spread of organisms initially inoculated into metaphyseal bone, through transphyseal vessels into the epiphysis and subsequently into the joint.<sup>303,304</sup> In addition to decompression of metaphyseal bone infection into the adjacent joint, erosion through the thin cortical bone may also occur, creating subperiosteal abscesses and subsequent involvement of the soft tissues of the extremity.<sup>297,303,305</sup> Virtually all reported cases have been caused by *S. aureus*, with recent reports now documenting the occurrence of CA-MRSA as a cause of neonatal osteomyelitis.<sup>302,306</sup>

The clinical presentation of neonatal bone and joint infections appears to take three general forms. First, the infection may be secondary to that of staphylococcal sepsis with bacteremia in which the focal bone and joint findings may not be the most prominent presenting symptom, but bone and joint sequelae of bacteremia become more apparent as the systemic infection is treated.<sup>189,302</sup> Second, and probably most common, an occult bacteremic inoculation of bone may occur, leading to either a single site, or in up to 50% of infants, multiple sites of infection.<sup>301,302,306</sup> In these infants, the clinical findings may be subtle, with signs

of irritability, with or without fever, with swelling around the affected bone and/or joint, and occasionally failure to move the limb (pseudoparalysis).<sup>302,307,308</sup> Risk factors for bacteremia in these infants include umbilical artery catheterization and prematurity.<sup>300-302,309</sup> In bacteremic disease, the femur and tibia are the most prominently involved bones, infected in approximately 80% of all cases of osteomyelitis.<sup>300-302</sup> Adjacent joints may be involved clinically in 25% to 50% of cases, although in an autopsy review of staphylococcal osteomyelitis, all infants with bone infection were documented on pathology to have adjacent arthritis.<sup>300,302-304</sup> Because most of the data are from small case series, accurate data on the presenting signs and symptoms may reflect strains circulating in a region at a particular point in time or may be related to outbreaks of specific strains.<sup>189</sup> Because of delays in the diagnosis of osteomyelitis, the location of the infection, which often involves bone on both sides of the physis and frequent involvement of the adjacent joint, late sequelae are common after bacteremic infection, with up to 50% of infants described to have long-term sequelae, including limb shortening, and decreased range of motion.<sup>299-301</sup>

A third clinical scenario leading to a bone or joint may be specifically linked to trauma. Osteomyelitis of the calcaneus has been documented to occur as a complication of heelstick blood sampling, most often presenting with focal swelling, erythema, and drainage.<sup>310</sup> Recent reports cite continuing problems secondary to a single heelstick that is used for metabolic screening in all newborns.<sup>311</sup> Fetal scalp monitoring has been associated with skull osteomyelitis.<sup>312</sup> Pyogenic arthritis of the hip is a reported complication of femoral vein venipuncture.<sup>313</sup>

Bone radiographs can show destructive changes in the bone, becoming apparent in the second week of infection. In the case of arthritis, widening of the joint space and bulging of the soft tissues may be seen as a clue to diagnosis. Ultrasonography may identify collections of pus that are subperiosteal or in the soft tissues. Although radionuclide bone scanning with technetium may provide additional useful information regarding the involvement of multiple bones, the normal increased vascularity of the neonatal metaphysis, may blur the differentiation between infection and increased uptake that is commonly seen in osteomyelitis in older children. Decreased blood flow from necrotic injury to the bone may lead to false-negative test results in the newborn. MRI is becoming the preferred imaging modality, based on excellent visualization of both soft tissues and bone, with a lack of ionizing radiation. However, MRI may be too sensitive in assessment of tissue inflammation in bones and soft tissues adjacent to the infected site, suggesting a greater extent of infection than is actually present. MRI with contrast provides additional information on inflammation in both bones and soft tissues and may be particularly helpful when imaging the spine to detect vertebral osteomyelitis/diskitis. CT imaging of neonatal bones and joints has a more limited role in diagnosis of acute infection.

## GASTROINTESTINAL

*S. aureus* is a common colonizer of the gastrointestinal tract of newborns, present in up to 93% of asymptomatic infants.<sup>314</sup> The prevalence of colonization is not surprising,



considering that large numbers of *S. aureus* can be recovered from samples of breast milk expressed from normal breasts of lactating and nonlactating women.<sup>315</sup> Recent metagenomic analyses indicate that in VLBW premature infants, meconium is not sterile and is less diverse from birth in infants who will develop nosocomial LOS, a phenotype severely worsened by prolonged empirical antibiotics.<sup>316</sup> Low diversity and redominance of *Staphylococcus* was seen in infants who developed sepsis, whereas healthy infants had more diversity and predominance of *Clostridium*, *Klebsiella*, and *Veillonella*.<sup>316</sup> Genetic studies confirm the gastrointestinal tract as a reservoir of CoNS associated with LOS.<sup>317</sup>

*S. aureus* disease in the gastrointestinal tract can be caused by one of the enterotoxins produced by the organism or can be a manifestation of bacterial invasion of the mucosa resulting in enterocolitis.<sup>318</sup> Although *S. aureus* has been described to colonize the gastrointestinal tract in the absence of clinical disease,<sup>314</sup> certain poorly defined risk factors may place colonized infants at risk of invasive disease, including feeding tubes and previous antibiotic therapy that may have facilitated colonization with *S. aureus*.<sup>319</sup> Clinical presentation includes signs and symptoms of generalized sepsis, in association with frequent, blood-tinged, thin, mucus-containing diarrheal stools. A recent report of neonatal staphylococcal enterocolitis, caused by MRSA, described a need for therapy with both intravenous and oral vancomycin to establish a microbiologic cure for both the systemic infection and colonization; the infant ultimately developed colonic stricture as a late complication of infection.<sup>320</sup> Delta-toxin-producing CoNS<sup>321</sup> and MRSA<sup>322</sup> have not been confirmed to play a major role in the pathogenesis of neonatal NEC, although they may play a supporting role in the disease process in some infants.

## Diagnosis

In the previously cited reports on clinical manifestations of staphylococcal infection, diagnosis is most often made by direct culture of the infected tissues or abscesses if the disease is focal, or by cultures of blood, urine, or CSF for diagnosis of sepsis/bacteremia, pyelonephritis, and meningitis/shunt infection, respectively. Organism identification and susceptibility testing are essential in understanding both the organism-specific severity of disease, as well as provide information on appropriate antimicrobial therapy. In addition, having the laboratory save the neonate's isolate allows one to compare subsequent episodes of infection by that organism in that infant or compare episodes of infection by the organism that may have spread to or from other neonates.

The diagnosis of infection by nonspecific laboratory tests that assess inflammation in the infant being evaluated can provide supportive evidence for infection. A detailed evaluation of specific tests, such as the total white blood count, the immature neutrophil (band-form) count, the mature:immature white blood cell ratio, the CRP, procalcitonin, cytokines<sup>323-327</sup> (IL-6, IL-8, IL-10, TNF- $\alpha$ ), and chemokines<sup>328,329</sup> (interferon- $\gamma$ -inducible protein-10, monocyte chemoattractant protein-1, RANTES [regulated on activation, normal T-cell expressed and secreted], epithelial neutrophil-activating peptide-78), is beyond the

scope of this chapter. The sensitivity, specificity, and positive predictive values vary with investigating institution and the population of neonates studied, with some laboratory test results increasing within a few hours of the onset of infection, whereas others may not increase for 1 to 2 days. Rather than a single test, a set of tests may offer the best hope for diagnosing early infection and tracking the response to therapy.<sup>329,330</sup> At the present time, CRP and procalcitonin appear to be among the most useful and the most widely available tests for assessment of neonatal sepsis. Some studies have suggested the usefulness of an elevated immature-to-total neutrophil ratio in identifying infants with CoNS septicemia.<sup>331-333</sup> No test has the ability to identify all infected infants, with the responsibility for decisions on further investigation and empirical antimicrobial therapy still requiring clinical judgment. The more premature and younger the infant, the greater is the interval from the time of infection to the time of a positive nonspecific test for inflammation. *S. aureus*, both MSSA and MRSA, appear to generate far more vigorous responses than CoNS. Therefore these nonspecific tests of inflammation cannot play a decisive role in the determination of whether a single positive blood culture for CoNS represents a true positive culture, or a contaminant.

Multiple positive blood cultures for the same strain of CoNS in a relatively asymptomatic infant may provide evidence of true infection that is more reliable than the white blood count or any cytokine concentration. Polymerase chain reaction techniques to detect 16S ribosomal RNA in plasma, followed by specific probes for *S. aureus* and CoNS, show promise but cannot currently be used as the sole diagnostic test for staphylococcal infection.<sup>334</sup> The evaluation of emerging nonculture-based diagnostic methodologies for neonatal infection are discussed in detail in Chapter 36.

## Antibiotic Treatment (see also Chapter 37)

### GENERAL PRINCIPLES

Optimal treatment for staphylococcal infections in neonates is designed to achieve both an appropriate antimicrobial exposure at the site of infection, as well as surgical control of the infection by drainage of any abscess and removal of any potentially infected foreign material. As with all neonatal bacterial infections, cultures of appropriate samples, based on signs and symptoms of infection, physical examination, and imaging, should provide the necessary information regarding the identity and susceptibility pattern of the pathogen.<sup>335</sup> The choice of empirical therapy, before susceptibility test results are known, depends on the local antibiotic resistance patterns for coagulase-positive and -negative staphylococci, the severity of infection, and the toxicity profile of the antibiotic for that infant.

For CoNS, susceptibility patterns are quite varied and are often based on the particular species isolated. For coagulase-positive strains, it is essential to know the susceptibility to  $\beta$ -lactamase stable penicillins. Culture and susceptibility information directly impacts selection of definitive therapy, allowing the use of the most narrow spectrum, least toxic antimicrobial regimens.

Although  $\beta$ -lactam agents are preferred for treatment of methicillin-susceptible staphylococcal infections in the neonate because of their bactericidal activity and overall safety, several other clinically useful classes may also show in vitro activity, including glycopeptides, aminoglycosides, lipopeptides, oxazolidinones, lincosamides, rifamycins, and trimethoprim-sulfamethoxazole (TMP-SMX). Unfortunately, as with so many other drugs for neonates, adequate prospective data on the safety and efficacy of these antimicrobials for the various tissue sites of infection caused by CoNS, MSSA, and MRSA are not available. Therefore extrapolation from other pediatric and adult data is necessary, with cautions for the neonate on both outcomes at dosages suggested, as well as on the safety of these antimicrobials.

Antimicrobial therapy of *S. aureus* infections should be separated into those that are susceptible to methicillin (MSSA) and those that are resistant (MRSA). Among MRSA, further differentiation should be made between the more antibiotic-resistant hospital-acquired strains (HA-MRSA) from those that are community acquired (CA-MRSA). No MRSA strains can be killed by penicillin or ampicillin, by the  $\beta$ -lactamase-stable, antistaphylococcal penicillins (methicillin, nafcillin, oxacillin, dicloxacillin), by the currently available cephalosporins (cefalexin, cephalothin, cefazolin, cefuroxime, cefotaxime, ceftriaxone), or by the carbapenems (meropenem, imipenem, ertapenem, doripenem). The HA-MRSA strains carry relatively large antibiotic resistance gene cassettes with concurrent resistance to clindamycin, macrolides (erythromycin, clarithromycin, and azithromycin), and aminoglycosides, a resistance profile that is usually not seen in CA-MRSA strains.

For mild-to-moderate invasive staphylococcal infections in neonates in areas of the world where methicillin resistance is still minimal, empirical therapy with first-generation cephalosporins (parenteral cefazolin, oral cephalexin) or antistaphylococcal penicillins (parenteral methicillin, oxacillin, nafcillin) is preferred. In some cases of mild skin infection, topical antibiotic therapy with mupirocin may suffice. For mild-to-moderate infections in those areas where CA-MRSA occurs at substantial rates (5%-10% or greater), clindamycin or vancomycin intravenously should be used empirically until susceptibility data are available. If clindamycin is used, caution should be exercised in treating erythromycin-resistant, clindamycin-susceptible strains of *S. aureus* with clindamycin, because those strains may display inducible clindamycin resistance (see "Clindamycin" later). The role of oral therapy for neonatal staphylococcal infections is not yet well defined. For MRSA strains that are susceptible, erythromycin, azithromycin, and clindamycin may be considered for mild infections, or step-down therapy in newborns who have responded well initially to intravenous therapy. TMP-SMX may be considered for mild infections in infants who no longer exhibit physiologic jaundice.

For serious infections in neonates in regions of the world in which CA-MRSA is routinely isolated, empirical therapy with vancomycin is preferred over clindamycin, given the bactericidal nature of killing and extensive experience with vancomycin in the newborn. For suspected *S. aureus* infections that are nosocomially acquired within institutions in which MRSA is present in other neonates, empirical therapy with vancomycin for presumed MRSA is also recommended. Furthermore, with data suggesting that the most

common pathogen responsible for bloodstream infections in LOS in hospitalized neonates is CoNS, most often resistant to  $\beta$ -lactam antibiotics, vancomycin is likely to provide effective therapy. For situations in which cultures demonstrate MSSA or methicillin- or penicillin-susceptible CoNS, it is imperative that therapy be switched back to traditional  $\beta$ -lactam antibiotics to minimize antibiotic pressure on staphylococcal species from vancomycin or clindamycin and thus to delay the emergence of resistance to these antibiotics.  $\beta$ -Lactam antibiotics are, in general, less toxic to the neonate, compared with vancomycin and clindamycin, and are better tolerated.

## VANCOMYCIN

Vancomycin is a first-generation, bactericidal glycopeptide antibiotic. Vancomycin normally inhibits growth of the organism by binding to cell wall precursors, inhibiting transglycosylase function, and cell wall synthesis. Complete resistance to vancomycin is relatively recent and quite limited, with the first cases of complete resistance being reported in 2002. However, of great importance is the observation that within every population of *S. aureus*, a very low frequency of vancomycin intermediately-resistant organisms exist, which may become selected in infants with prolonged exposure to vancomycin.<sup>335</sup> By contrast, complete vancomycin resistance is still exceedingly rare and created by a different mechanism that parallels vancomycin resistance in enterococci.

Dosing of vancomycin is classically designed to achieve an area under the curve:minimal inhibitory concentration (AUC:MIC) ratio of greater than or equal to 250, and this is associated with microbiologic cure in experimental in vitro and in vivo animal models, as well as in retrospective analyses of infections in adults. In neonates, many dosing recommendations exist, including both intermittent dosing as well as continuous infusion, primarily based on chronologic and gestational age, as well as based on serum creatinine.<sup>336-341</sup> An initial loading dose of 15 mg/kg is most often recommended,<sup>342</sup> followed by repeated dosing every 8 to 24 hours, reflecting longer dosing intervals for the youngest, most premature infants. Each dose should be administered over 60 minutes. In neonates, data on continuous infusion of vancomycin are scant, and there is no randomized controlled study available on the efficacy and safety of continuous or intermittent infusions.<sup>342</sup> Close monitoring of renal function and serum concentrations of vancomycin are recommended in all neonates receiving therapy, allowing for adjustment of vancomycin dosing regardless of the initial empirical dosing regimen chosen. Intraventricular vancomycin has been used to treat CNS infections, primarily ventriculoperitoneal shunt infections caused by CoNS,<sup>294,295</sup> although CSF concentrations may be therapeutic after intravenous administration.<sup>337</sup> Newer analysis in the pediatric population suggests that targeted exposure using the vancomycin AUC:MIC, compared with trough concentrations, is a more realistic target in children. Depending on age, serum creatinine, and MIC distribution, vancomycin at a dosage of 60 to 70 mg/kg/day was necessary to achieve AUC:MIC greater than or equal to 400 in 75% of patients<sup>343</sup>; such monitoring requires Bayesian estimation based on both peak and trough levels to ensure accuracy in predicting drug exposure.<sup>344</sup>

## CLINDAMYCIN AND ERYTHROMYCIN

Both clindamycin, a lincosamide, and erythromycin, a macrolide, inhibit ribosomal function and produce a primarily bacteriostatic effect by binding to sites on the ribosome. Most strains of MSSA remain susceptible to clindamycin and erythromycin; many strains of CA-MRSA remain susceptible, but most strains of HA-MRSA and CoNS are resistant to these antibiotics. Staphylococcal resistance to erythromycin may occur by two mechanisms: by methylase-mediated dimethylation of the 23S ribosomal binding site of the macrolides and by the presence of an efflux pump that expels the macrolide from the intracellular environment of the pathogen. The methylase gene *erm* is usually inducible, but in any large population of organisms, mutants will occur that constitutively produce methylase, providing complete resistance to all macrolides (erythromycin, azithromycin, clarithromycin), clindamycin, and the streptogramins (quinupristin/dalfopristin). By contrast, the most prevalent macrolide efflux pump for staphylococci, *msrA*, does not recognize, bind to, or eliminate clindamycin from within the bacteria, allowing these strains to remain susceptible to clindamycin. Any strain that demonstrates *in vitro* erythromycin resistance and clindamycin susceptibility must also be tested for methylase-mediated clindamycin resistance by an additional assay, the D-test. Current laboratory reporting guidelines suggest that hospitals report erythromycin-resistant, D-test–positive strains as clindamycin resistant, on the basis of reported clinical failures of clindamycin in treating infections caused by inducible organisms. Because the true clinical significance of inducible *erm*-mediated resistance for clindamycin is not well defined at present, it is prudent to use other antibiotic options for the seriously ill neonate with infection caused by a D-test positive strain.

Erythromycin is associated with the occurrence of pyloric stenosis in the newborn infant,<sup>345</sup> a side effect that is likely to be less prevalent in clarithromycin and azithromycin. Clindamycin, erythromycin, and azithromycin are available in oral and intravenous formulations, but little prospective, comparative data exist for their use in the neonate.

## LINEZOLID

Of the newer approved antibiotics with activity against MRSA, linezolid is the only one currently approved by the United States Food and Drug Administration (FDA) for use in the neonate.<sup>346</sup> As might be predicted, resistance to linezolid has been documented to develop in adults receiving therapy for a bacteremic MRSA infection, although to date, resistance remains rare.<sup>347</sup> Linezolid is an oxazolidinone-class protein synthesis inhibitor, the first of this new class of antibiotics. Linezolid is a ribosome-inhibiting, bacteriostatic agent, active against both *S. aureus* and CoNS.<sup>348</sup> Data on pharmacokinetics are available for all pediatric age groups, including premature neonates younger than 34 weeks of age. Linezolid can be administered both intravenously and orally, with virtually 100% of the agent absorbed by the oral route. Protein binding in plasma is approximately 30%, and the drug is well distributed in tissues. Linezolid is cleared by the kidneys, both unchanged and after oxidation of the parent compound. Because oxidation of linezolid is not dependent on renal function, no dose reduction is needed for

renal insufficiency. Linezolid has been studied in neonates and older children for nosocomial and community-acquired pneumonia and for complicated and uncomplicated skin and skin structure infections.<sup>349,350</sup> The clinical response rates for each of these tissue specific infections were equivalent to comparator agents, usually vancomycin. The pathogen-specific response rates for infections caused by *S. aureus*, for both methicillin-sensitive and methicillin-resistant strains, and response rates in infections caused by coagulase-negative staphylococci were also statistically equivalent to vancomycin. Similarly, the rates for clinical and laboratory adverse events were equivalent to those in vancomycin-treated control patients. In neonates and children enrolled in these registration trials, the hematologic toxicity profiles for both neutropenia and thrombocytopenia were equivalent to vancomycin.<sup>348</sup> These data suggest that hematologic toxicity of thrombocytopenia and neutropenia seen in adults may not be seen as frequently in neonates and children.

Recommendations for the dosage regimen for preterm neonates less than 7 days of age (gestational age younger than 34 weeks) are based on data from registration trials involving very few neonates. Preterm neonates should be initially given 10 mg/kg every 12 hours. For neonates with a poor response to infection caused by a susceptible organism, an increased dose of 10 mg/kg every 8 hours can be provided. However, by 7 days of age, all neonates, regardless of gestational age, should receive 10 mg/kg every 8 hours. The interpatient variability in neonates was noted to be greater than that seen in adults and may reflect variation in the rate of maturation of mechanisms of elimination.

Unfortunately, in studies of CSF, linezolid concentrations in infants with ventriculoperitoneal shunts receiving systemic therapeutic dosing, adequate concentrations were not consistently achieved. Although a case report exists for the treatment of a staphylococcal CNS infection in a neonate,<sup>296</sup> the routine use of linezolid for the treatment of CNS infections cannot be recommended at this time. Similarly, case reports on the treatment of neonatal endocarditis caused by MRSA exist, but the safety and efficacy of linezolid for this indication remains to be defined. The role of combination therapy using linezolid is also not defined. A new anti-staphylococcal oxazolidinone, tedixolid (Sivextro, Cubist Pharmaceuticals, Lexington, Mass), was approved by the FDA in 2014 for use in adults, but has yet to be studied in the newborn population.

## DAPTOMYCIN

An antibiotic approved only for use in adults, daptomycin is a novel lipopeptide bactericidal agent for gram-positive organisms, including *S. aureus* and CoNS. Structurally, daptomycin is a 13–amino acid cyclic peptide with a lipophilic tail that inserts into the cell membrane, leading to depolarization of the membrane; inhibition of protein, DNA, and RNA synthesis; and cell death. Daptomycin shows concentration-dependent killing pharmacodynamics. It is available only in an intravenous formulation. The prolonged half-life in adults of 8 to 9 hours allows for once-daily dosing. The antibiotic is highly protein bound (90%) and is excreted primarily by the kidney with little degradation of the parent compound. In renal insufficiency, the dose is decreased according to the degree of renal failure. In adults, daptomycin is approved by

the FDA for the treatment of complicated skin and skin structure infections (caused by *S. aureus*, including MRSA), as well as for bacteremia and endocarditis. Daptomycin also shows in vitro activity against vancomycin-resistant *S. aureus* and should represent an effective agent should these strains become more widespread. Daptomycin is not indicated for the treatment of pneumonia because surfactant binding to the antibiotic is associated with inactivation. A recent study of single-dose pharmacokinetics in young infants showed clearance similar to older children and higher than seen in adolescents and adults.<sup>351</sup> Neurologic toxicity has been demonstrated in a neonatal beagle pup animal model at doses similar to those proposed for human infants, suggesting that daptomycin not be used in neonates until additional data are available. Myopathy is a potential adverse event noted in early phase I studies but with once-daily dosing in adults, and in the preliminary newborn data,<sup>351</sup> no muscle toxicity (elevated creatinine phosphokinase) was documented.

### QUINUPRISTIN-DALFOPRISTIN

The streptogramins are antibiotic derivatives of natural products of *Streptomyces pristinaespiralis*. Two of the streptogramins, quinupristin and dalbopristin, when used together in a fixed combination, have been shown to be bactericidal against many gram-positive organisms, including staphylococci and certain enterococci. Each antibiotic is bacteriostatic, but, when used together in a 30:70 ratio, the combination is bactericidal. The combination is approved by the FDA as Synercid for adults, for the treatment of vancomycin-resistant *Enterococcus faecium* infections and for the treatment of skin and skin structure infections caused by *S. aureus* (only methicillin-susceptible strains were isolated from study patients). In vitro, quinupristin-dalbopristin is also active against MRSA and vancomycin-resistant *S. aureus*, although no clinical data are available for treatment of these infections. Quinupristin-dalbopristin is available only in an intravenous preparation. Both drugs are primarily eliminated through biliary excretion, with minimal metabolism. Inflammation and pain at the infusion site are substantial problems. Furthermore, many mechanisms of bacterial resistance have been documented, ultimately limiting the clinical usefulness of this combination.

### COMBINATION ANTIMICROBIAL THERAPY

Although many combinations of antibiotics have been used in adults, few have been studied prospectively, with virtually no prospective comparative evaluations available for children and neonates. For invasive *S. aureus* disease, infective endocarditis in adults has resulted in some of the highest mortality rates, resulting in guidelines that recommend aggressive combination therapy based on animal model in vitro data, data from CoNS infections, and in the absence of human data for *S. aureus*.<sup>352</sup> For MSSA endocarditis, combination therapy with a  $\beta$ -lactam penicillin (oxacillin or nafcillin), with the addition of rifampin plus the addition of gentamicin for the first 2 weeks of therapy, is believed to result in optimal microbiologic efficacy. For MRSA, vancomycin plus rifampin, with gentamicin for the first 2 weeks of therapy, should be considered.<sup>352</sup> A report on vancomycin plus rifampin combination therapy of persisting CoNS bacteremia after removal of a central catheter provides some support to this approach.<sup>353</sup>

A Cochrane review of intravenous immunoglobulin therapy of suspected or documented neonatal sepsis evaluated nine clinical trials. Although substantial heterogeneity existed across studies in immune globulin preparations, dosing regimens, and populations studied, no substantial benefit was derived from treatment, particularly with respect to mortality in infants with either documented infection suspected or subsequently proven infection.<sup>354</sup>

### CATHETER REMOVAL

The decision to remove an indwelling catheter from a neonate with bacteremia often is difficult, especially when securing subsequent intravascular access may be challenging. Delayed removal of a central catheter in the setting of bacteremia may be associated with an increased risk of infection-related complications.<sup>214</sup> For infants with CoNS bacteremia, successful treatment of bacteremia may be possible with the central venous catheter in situ.<sup>214</sup> However, if bacteremia persists for longer than 4 days, the chance for subsequent clearance is reduced,<sup>213</sup> and the risk of end-organ damage may be increased.<sup>190,214</sup> The presence of a ventricular reservoir or ventriculoperitoneal shunt increases the chance of the development of meningitis in the setting of prolonged catheter-related bacteremia. Thus prompt removal of an indwelling central venous catheter should be considered in infants with CNS hardware.<sup>213</sup> A relationship between the dwell time of peripherally inserted central catheters and risk of line-associated bacteremias has been calculated,<sup>355</sup> so the true need for central access in support of individual patients should be assessed daily.

## Prevention

### HYGIENIC MEASURES

Major efforts to prevent staphylococcal infections in neonates, rather than being required to treat them, are of great value. General principles underlying nosocomial infection in the NICU and measures to reduce their occurrence that apply broadly to staphylococcal infections are discussed in detail in Chapter 35. Some specific considerations relevant to staphylococci are discussed briefly here.

Staphylococci may be spread through the fomites; thus overcrowding of infants in an NICU may increase the risk of colonization and the potential for disease. In an outbreak situation, attempts to control the spread of staphylococci through remediation of overcrowding and isolation of infected or colonized patients have been shown effective in helping to curtail the outbreak, even in the case of MRSA.<sup>356</sup>

A primary determinant of infant colonization is nursing care. Maintaining an appropriate nurse-to-infant ratio is an important factor in reducing disease once a disease-associated *S. aureus* strain gains entrance to a nursery, especially in an NICU.<sup>357</sup> In addition, there are a variety of preventive maneuvers directed at those with direct infant contact, including frequent mask, gown, and glove changes before handling infants<sup>358,359</sup>; application of antimicrobial or antiseptic ointment or spray<sup>360,361</sup>; and elimination of carriers from the nursery area.<sup>362,363</sup> In some situations, control of an epidemic requires removal of the nurse carrier from the nursery.<sup>364</sup>

Currently, the Centers for Disease Control and Prevention recommends contact isolation for patients colonized or infected with MRSA.<sup>365</sup> This practice was shown to reduce nosocomial transmission of MRSA by 16-fold during an outbreak of MRSA in a NICU.<sup>366</sup> Several recent publications have focused on nursery infection control measures, documented to be effective in preventing the entry of CA-MRSA into a nursery, and its spread within the nursery.<sup>367-370</sup>

In the early 1960s, attempts were made to stop virulent *S. aureus* epidemics in 10 NICUs throughout the United States by using the technique of bacterial interference.<sup>371,372</sup> This technique involved deliberate implantation of an *S. aureus* strain of low virulence (502A) on the nasal mucosa and umbilicus of newborns to prevent colonization with the virulent *S. aureus* strain. Although this procedure was successful in curtailing epidemics,<sup>373</sup> it is not widely used or recommended currently.

Proper hand hygiene among nursery health care providers is a fundamental factor in reducing colonization rates. Mortimer and associates<sup>374</sup> achieved a reduction in infant colonization from 92% to 53% by insisting that attendants wash their hands. Proper education and monitoring of hand-hygiene practices are critical to the effectiveness of this intervention.<sup>375,376</sup> Hands must be cleansed before and after patient contact or contact with equipment that is used for patient care. Hands also should be cleansed after glove removal. Proper hand hygiene involves applying alcohol-based waterless rubs if hands are not soiled,<sup>377</sup> or washing the hands for at least 10 to 15 seconds with either chlorhexidine gluconate or triclosan hand-washing agents.<sup>378</sup>

With the rise in prominence of CoNS as nosocomial pathogens, strategies for disease prevention have become increasingly important. As with *S. aureus*, strict hand hygiene is of primary importance in minimizing staff-to-patient and patient-to-patient spread of CoNS. In addition, meticulous surgical technique to limit intraoperative bacterial contamination is critical in minimizing infection related to foreign bodies. Strict attention to protocols for the insertion and management of intravenous and intraarterial catheters may decrease the risk of catheter-related infections.<sup>379</sup> In patients who require intravenous access for prolonged periods of time, percutaneous placement of a small diameter silastic catheter is preferred when possible. In one study, these catheters were maintained for as long as 80 days, with an infection rate of less than 10% in infants weighing less than 1500g.<sup>380</sup>

## ANTIBIOTIC PROPHYLAXIS

Investigational therapies to reduce neonatal bacteremia caused by staphylococci have been directed at the use of antibiotic prophylaxis, for instance, antibiotic-impregnated devices. Given the large burden of CoNS catheter infections in premature infants, investigations of prophylactic antibiotics to prevent infection were undertaken by a number of institutions, as recently reviewed.<sup>381-385</sup> Vancomycin was documented to be successful in significantly decreasing the rate of suspected or documented sepsis caused by CoNS. Antibiotic-based methods to prevent bacteremic infection have included the use of a vancomycin solution (25 µg/mL) to dwell inside the infant's central venous catheter two to three times daily for up to 60 minutes<sup>384</sup>; the administration of low-dose vancomycin at 5 mg/kg twice daily<sup>383</sup>; or

the addition of vancomycin to hyperalimentation solutions to a concentration of 25 µg/mL for routine administration. Although all three methods were successful at decreasing episodes of sepsis, the overall mortality in treatment versus control groups was not affected. Because of concerns for the emergence of vancomycin-resistant organisms, routine use of prophylactic vancomycin for all neonates at risk of CoNS bacteremia is not currently recommended. Potential risks associated with prophylactic vancomycin, including ototoxicity, nephrotoxicity, and selection for resistant bacteria, have not been well evaluated.

## IMMUNE PROPHYLAXIS

Studies evaluating the effectiveness of immune globulin preparations have, in general, not documented convincing, substantial benefits for the populations of premature infants studied.<sup>386</sup> This may, however, reflect the lack of effectiveness of a specific biologic preparation or suggest that particular subpopulations may benefit more from treatment than others, rather than proving the immune globulins have no potential role in prophylaxis or treatment. Other polyclonal antibody approaches to prophylaxis in premature infants have used high-titer anti-*S. aureus* immune globulin (Alta-staph; Nabi Biopharmaceuticals, Rockville, MD), prepared from adult volunteers immunized with a staphylococcal vaccine. Pharmacokinetic, safety, and clinical outcome data in neonates randomized to receive either immune globulin or placebo did not show benefit in the limited trials performed to date.<sup>386,387</sup>

Studies of monoclonal antibodies directed against specific staphylococcal epitopes are ongoing. A randomized, placebo-controlled trial was recently conducted in premature infants to prevent staphylococcal infection, using an intravenous immunoglobulin preparation selected from donors with high activity against specific staphylococcal fibrinogen-binding protein, clumping-factor A, and Ser-Asp dipeptide repeat G (INH-A21 [Veronate]; Inhibi-tex, Alpharetta, Ga). No benefit to prophylaxis was noted in the recipients of this staphylococcal-specific immune globulin.<sup>388,389</sup> An antistaphylococcal monoclonal antibody, BSYX-A110 (Biosynexus, Gaithersburg, Md), has been developed for the prevention of CoNS sepsis. This antibody targets staphylococcal lipoteichoic acid and has been shown to be safe and well tolerated when administered by intravenous infusion to high-risk neonates.<sup>390</sup> The efficacy of any monoclonal antibody therapeutics in preventing CoNS infections and related morbidity and mortality has not been established and, as such, they are not currently recommended.<sup>391</sup>

Lactoferrin is an iron-binding glycoprotein present in breast milk that is believed to contribute to innate antibacterial immunity of the intestinal barrier through a combination of restricting pathogen access to iron, cell wall lytic activity of its component peptides, and promotion of epithelial barrier maturation.<sup>392</sup> Human recombinant lactoferrin may synergize with vancomycin and nafcillin in terms of in vitro activity against CoNS.<sup>393</sup> A recent randomized study of bovine lactoferrin supplementation in VLBW premature infants demonstrated a promising reduction in the rate of LOS in the treatment group (risk ratio, 0.34; 95% confidence interval, 0.17 to 0.70).<sup>394</sup>

## Conclusion

Staphylococcal infections result in significant morbidity and mortality in the neonate. Although CoNS are frequent causes of less severe infections, the continuing relatively high rate of community-associated and hospital-associated infections caused by more aggressive *S. aureus* and the recent emergence of CA-MRSA with exceptionally high mortality rates has created an unprecedented need to understand the biology and mechanisms of virulence of staphylococci. In this way, we can generate improved approaches to both prevent and treat infections. A profound need exists to develop more safe and effective antimicrobials and immune therapies to mitigate the substantial morbidity and mortality caused by these pathogens.

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