CHAPTER 14

STAPHYLOCOCCAL INFECTIONS

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CHAPTER OUTLINE

Staphylococcal disease has been recognized in neonates for centuries; it was reported in 1773, when pemphigus neonatorum was described [1]. Outbreaks of staphylococcal disease in nurseries were first noted in the late 1920s [2], and the memorable term "cloud baby" was subsequently coined to describe index cases, often asymptomatic, who contaminated the nursery atmosphere with *Staphylococcus aureus* colonizing their respiratory tract, skin, or umbilical cord [3]. Until the late 1970s, staphylococcal disease in newborn infants was caused most often by *S. aureus* [4]. In recent decades, coagulase-negative staphylococci (CoNS) have assumed an equally important role, especially in premature infants in neonatal intensive care units (NICUs) [5–7], often responsible for 50% 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 infant population, and obstetric and nursery practices. Other factors certain to influence transmission include virulence properties of the individual *S. aureus* strains and often poorly defined immunogenetic host factors. The complexity of isolating and investigating each variable in the epidemiologic equation accounts for the disagreement in the literature about which factors predominate in transmission and prevention of staphylococcal disease. A particular factor that is critical in one epidemic may not be a driving factor under different circumstances.

Quantitative culture studies show that very few *S. aureus* organisms are capable of initiating colonization in the newborn. Less than 10 bacteria can establish umbilical colonization in 50% of newborns, whereas approximately 250 organisms can achieve a similar effect on the nasal mucosa [8]. Colonization of the newborn umbilicus, nares, and skin occurs early in life. By the 5th day in the nursery, the colonization rate among nursery inhabitants

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may be 90% [9]. The umbilicus or rectum usually is colonized before the nares [10,11].

These findings provide a plausible explanation for the challenge of defining any single factor in the environment (e.g., fomites, hands, clothes) as the ultimate source of infection. Nevertheless, most evidence indicates that the initial and perhaps major source of infection is medical and nursing personnel [8]. A strain of *S. aureus* common among medical attendants is far more likely than a maternal strain to colonize a given infant in the nursery [12]; in 85% of cases, infant colonization with *S. aureus* is likely to originate from an attendant's touch [13]. Persons with overt cutaneous lesions or disease often are highly infectious, but asymptomatic carriers can be infectious also [14], and carriage on the skin, in the anterior nares, and in the perineal area is relevant [15,16]. The frequency of intestinal carriage of the pathogen may be greatly underestimated as well [17].

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

The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network reported that from 1998-2000, approximately 8% of initial episodes of late-onset sepsis among infants with very low birth weight (<1500 g) were caused by *S. aureus* [6]. More recently, Carey and colleagues [23] reported the epidemiology of MSSA and MRSA IFSA 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. Overall, the clinical presentations and the crude mortality rates (16% to 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 (31%); bacteremia plus skin and soft tissue (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 very low birth weight infants; most infections in infants weighing more than 2500 g were associated with surgical procedures. Reports of small outbreaks of CA-MRSA in NICUs and well-infant nurseries are appearing with increasing frequency [24–26].

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 [27]. In these situations, identity of the strain 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 [28]. The discriminatory power of this approach is less than that of PFGE, so the usefulness for the evaluation of local outbreaks is less [29]. Nevertheless, MLST allows the user to compare sequences from isolates of various locations through a central database (http://www.mlst.net).

COAGULASE-NEGATIVE STAPHYLOCOCCI

CoNS 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 [30]. With sensitive culture techniques, the nose, umbilicus, and chest skin are found to be colonized with CoNS in 83% of neonates by 4 days of age [31]. Rates of colonization with *S. epidermidis* in one study of infants in a NICU were as follows: nose, 89%; throat, 84%; umbilicus, 90%; and stool, 86%; simultaneous percentages for *S. aureus* were 17%, 17%, 21%, and 10% [30]. Although most infants acquire CoNS from environmental sources, including hospital personnel, a small percentage are colonized by vertical transmission [32,33]. Isolates of *S. epidermidis* and other CoNS resistant to multiple antibiotic agents are common. In a study involving premature neonates, D'Angio and associates showed that the incidence of strains resistant to multiple antibiotics increased from 32% to 82% by the end of the 1st week of life [34].

The observation that CoNS are important nosocomial pathogens among newborns, especially low birth weight 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 and ventriculoperitoneal shunts, and use of feeding tubes. In more recent epidemiology, CoNS account for more than half of bloodstream isolates obtained from neonates with late-onset sepsis [5–7].

An inverse relationship exists between the rate of infection with CoNS and birth weight and gestational age. Additional risk factors that are associated with CoNS bacteremia among very low birth weight neonates include respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, severe intraventricular hemorrhage, and necrotizing enterocolitis [6].

Certain nutritional factors are associated with the development of late-onset sepsis, including delayed initiation of enteral feeding, prolonged period to reach full enteral feeding status, delayed reattainment of birth

weight, and prolonged parenteral hyperalimentation [6]. In a case-control study, administration of intralipids through a polytetrafluoroethylene (Teflon) catheter was also shown to be associated with an increased risk of bacteremia caused by CoNS [35]. Most experts believe 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 very low birth weight premature infants, with compromised immunity, are surviving for longer periods.

MICROBIOLOGY

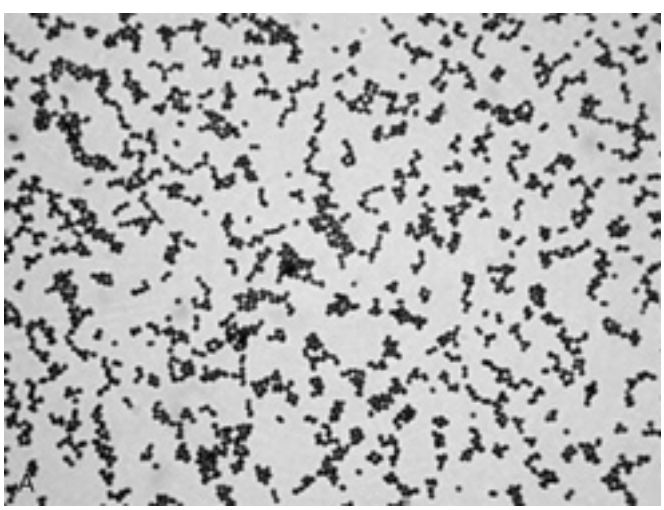
Staphylococci are members of the family Staphylococcaceae and are nonmotile, non–spore-forming bacteria that are catalase-negative. Species of staphylococci are separated into two large groups on the basis of ability to produce the extracellular enzyme coagulase. Organisms that produce coagulase are known as coagulase-positive staphylococci, or *S. aureus* [36], 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 are capable of growing under anaerobic conditions as well. They grow readily on most routine laboratory media, including Luria broth, and usually are isolated from clinical specimens using sheep blood agar. Gram staining reveals gram-positive cocci 0.7 to 1.2 mM in diameter that are 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. 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. Colonies of *S. aureus* often are deep yellow or golden in color and typically are surrounded by a zone of β-hemolysis (Fig. 14–1B). By contrast, colonies of CoNS usually are chalk-white, often lacking surrounding hemolysis.

STAPHYLOCOCCUS AUREUS

For clinical purposes, many of the key characteristics of *S. aureus* can be determined by simple procedures performed with commercial rapid identification kits and automated systems [36]. Historically, phage typing and serologic typing were the most common systems for differentiating strains of *S. aureus* for epidemiologic purposes [37]. In contemporary analysis, molecular approaches such as PFGE and MSLT have become the standard for defining strain identity in a patient with multiple isolates or in a possible outbreak involving multiple patients [38,39].

The staphylococcal cell wall is composed of two major components, peptidoglycan and teichoic acid [40,41]. *S. aureus* peptidoglycan is composed of chains of acetylglucosamine, acetylmuramic acid, alanine, glutamic acid, and lysine or diaminopimelic acid, with pentaglycine bridges that cross-link these chains. Four penicillin-binding proteins called PBP1, PBP2, PBP3, and PBP4 play an important role in peptidoglycan biosynthesis and are inactivated by β-lactams [42]. A mutated form of PBP2 (PBP2a) encoded by the *mecA* gene is the basis of methicillin resistance in the current epidemic of hospital-acquired MRSA (HA-MRSA) and CA-MRSA disease. 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 [43]. Antibodies to teichoic acid enhance opsonophagocytic killing of nonencapsulated strains of *S. aureus*, but have little effect on encapsulated isolates [44]. In contrast, antibodies to peptidoglycan play a key role in the opsonization of encapsulated *S. aureus* [45].



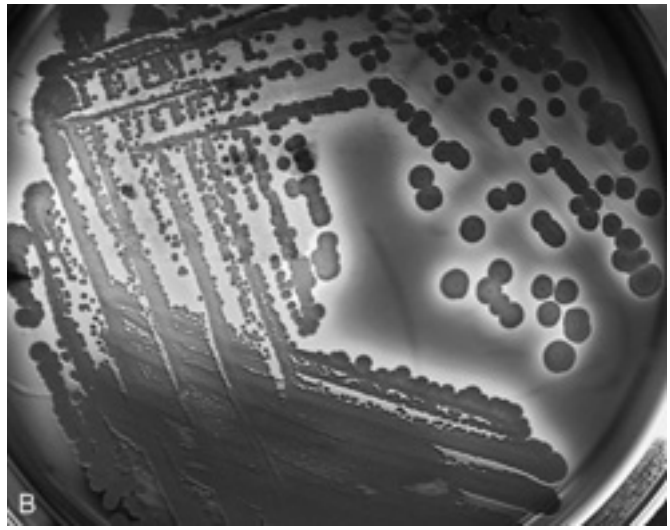


FIGURE 14–1 **A**, Gram stain of *Staphylococcus aureus* showing characteristic clusters. **B**, Blood agar plate showing growth of *S. aureus* with zone of β-hemolysis surrounding colonies.

Antibodies to *S. aureus* teichoic acid and peptidoglycan are widespread in screens of the human population [45].

In addition to peptidoglycan and teichoic acid, other components of the *S. aureus* cell wall include the group antigen known as protein A, an immunoglobulin Fc binding protein, and numerous 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) [46,47]. 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 [47]. These include several proteins involved in extracellular matrix binding and promoting *S. aureus* adherence to host epithelium [48].

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 [49]. The serotype 5 *S. aureus* capsule has the structure (?4)-3-O-Ac-β-d-ManNAcA-(1?4)-a-l-FucNAc-(1?3)-β-d-FucNAc-(1?)$_n$, whereas the serotype 8 capsule has the structure (?3)-4-O-Ac-β-d-ManNAcA-(1?3)-a-l-FucNAc-(1?3)-β-d-FucNAc-(1?)$_n$ [50,51]. Although these two capsular polysaccharides differ only in the sugar linkages at 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 clearance.

Small colony variants of *S. aureus* isolated from clinical specimens have been recognized for nearly a century. Small colony variants have now been linked to persistent and relapsing *S. aureus* infections, including chronic osteomyelitis and soft tissue abscesses [52,53]. These phenotypes can be traced to biochemical defects in electron transport, which are associated with slow growth and reduced α-toxin production that 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. Because they can be overlooked in the laboratory owing to their fastidious growth, extra efforts to identify small colony variants should be undertaken in the setting of persistent or relapsing *S. aureus* infection despite antibiotic therapy [52,53].

Nucleotide sequencing of the whole genome for several isolates of *S. aureus* [54,55], including MRSA strains [56,57], has established that the genome is 2.8 to 2.9 Mb in size, with approximately 2600 to 2700 open reading frames and an overall guanine-to-cytosine content of approximately 33% [54,55]. Much of the *S. aureus* genome seems to have been acquired by lateral gene transfer [56]. 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. The exotoxin and enterotoxin islands are closely linked to other gene clusters encoding putative virulence factors.

COAGULASE-NEGATIVE STAPHYLOCOCCI

CoNS are a heterogeneous group of organisms that have been divided into 32 species [36]. The following 15 species of CoNS are found as members of the normal human flora: *S. epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, *Staphylococcus capitis*, *Staphylococcus warneri*, *Staphylococcus hominis*, *Staphylococcus xylosus*, *Staphylococcus cohnii*, *Staphylococcus simulans*, *Staphylococcus auricularis*, *Staphylococcus saccharolyticus*, *Staphylococcus caprae*, *Staphylococcus pasteuri*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi* [36,58]. Among these species, several occupy very specific niches on the skin. *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 [36]. Differentiation of two strains belonging to the same species (subspeciation) represents a more difficult problem, however. Analogous to the situation with *S. aureus*, contemporary techniques for distinguishing strains of a given species include PFGE and MLST [59]. 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 open reading frames, greater than 10% smaller than the published genomes of *S. aureus* isolates [60]. Compared 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-mediated 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, contributing to persistence and facilitating spread within normally sterile sites of the host.

EPITHELIAL ATTACHMENT AND INVASION

S. aureus initiates adherence by binding to components of the extracellular matrix of the host. This adherence is mediated by protein adhesins known as MSCRAMMs

(*m*icrobial *s*urface *c*omponents *r*ecognizing *a*dhesive *m*atrix *m*olecules), 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 [48]. *S. aureus* MSCRAMMs can promote binding to fibronectin, fibrinogen, 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. *S. aureus* also expresses the fibrinogen-binding proteins, or "clumping factors," ClfA and ClfB [61,62]. Each Clf protein recognizes a different part of the fibrinogen model and could synergize 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 [63]. In rat endocarditis studies, ClfA mutant *S. aureus* have reduced virulence [64]. Finally, the collagen-binding MSCRAMM Cna allows *S. aureus* to adhere to collagenous tissues such as cartilage [65]. In a murine septic arthritis model, a Cna-null mutant strain of *S. aureus* was significantly attenuated for virulence [66]. The *ica*ADBC-encoded polysaccharide intercellular adhesin (PIA) and polymeric *N*-acetylglucosamine contribute to *S. aureus* biofilm development [67]; these genes and resultant phenotype shared by *S. epidermidis* are discussed in more detail subsequently.

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 [68,69]. Additionally, positively charged lysyl-phosphatidylglycerol

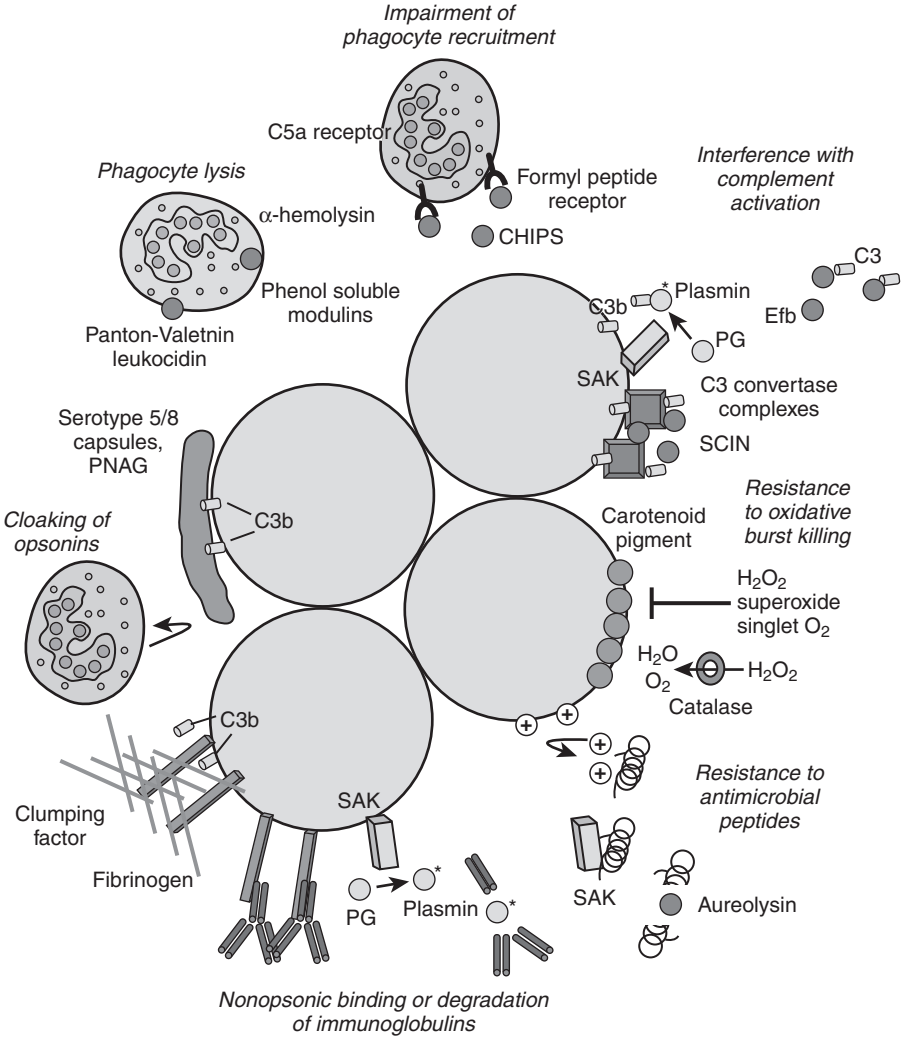


FIGURE 14–2 ***Staphylococcus aureus* possesses multiple virulence mechanisms to resist clearance by host phagocytic cells.** Phagocyte recruitment is restricted by "chemotaxis inhibitory protein of staphylococci" (CHIPS) binding to chemokine receptors. Complement activation is blocked by protein Efb binding of soluble C3 and inhibition of the 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 and inactivation by staphylokinase (SAK). Protein A binds Fc domains of immunoglobulins in a nonopsonic manner, whereas fibrinogen-binding clumping factor and the surface polysaccharide capsule and polymeric *N*-acetylglucosamine (PNAG) cloak surface-bound opsonins from phagocyte recognition. The heptameric pore-forming toxins α-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.

modifications of teichoic acids are encoded in the functions the *S. aureus mpr*F or *lys*C genes and contribute to human antimicrobial peptide resistance [70,71]. *S. aureus* mutants defective in Dlt or MprF show reduced virulence in small animal infection models [69,72]. 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 [73,74].

Many *S. aureus* strains produce the "chemotaxis inhibitory protein of staphylococci" that binds with high avidity to the leukocyte receptors for C5a and *N*-formyl peptides, blocking functional engagement of the respective chemoattractants and delaying neutrophil recruitment to the site of infection [75]. *S. aureus* also expresses the extracellular adherence protein that binds and inhibits intracellular adhesion molecule 1, the endothelial receptor required to initiate leukocyte adhesion and diapedesis [76].

S. aureus expresses multiple factors to interfere with host complement-mediated clearance [77]. Cleavage of C3 to opsonically active C3b is accomplished after assembly of C3 convertase complex C4bC2a (classical/lectin pathways) or C3bBb (alternative pathway) on the bacterial surface. The secreted approximately 10 kDa *S. aureus* protein known as staphylococcal complement inhibitor 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 [78]. The surface-anchored *S. aureus* fibrinogen-binding protein ClfA recruits fibrinogen to the bacterial surface in a fashion that impairs complement deposition [79,80]. The secreted *S. aureus* fibrinogen-binding protein Efb-C can bind free C3, altering the solution conformation of this crucial complement component such that it is unable to participate in its downstream opsonization functions [81]. Finally, another mechanism of interference with complement opsonization derives from bacterial coaptation of host proteolytic activities. The *S. aureus* surface receptor staphylokinase binds plasminogen from host serum and converts zymogen to the active protease, plasmin. Surface bound plasmin can cleave human C3b and C3bi from the bacterial cell wall and impair neutrophil phagocytosis [82].

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 [83]. This Fc-binding activity is classically associated with protein A of *S. aureus*, which serves to block Fc receptor–mediated phagocytosis and contributes to animal virulence [84]. In addition, most *S. aureus* clinical isolates express surface capsules composed of serotype 5 or 8 polysaccharide [49]. 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 [85,86]. Analogous functions can be ascribed to an additional *S. aureus* surface polysaccharide, polymeric *N*-acetylglucosamine [87]. Neither of the *S. aureus* exopolysaccharides directly inhibits deposition of complement factors on the bacterial surface; rather, they seem to serve as a superficial "cloak" that restricts access of phagocytes to the opsonins [83].

Catalase production is a diagnostic tool to distinguish staphylococci from streptococci in the clinical laboratory, and the ability of staphylococcal catalase to detoxify hydrogen peroxide generated during oxidative burst may promote phagocyte resistance and virulence [88]. The golden pigment for which *S. aureus* is named is a carotenoid molecule with potent antioxidant properties that is necessary and sufficient to promote bacterial neutrophil resistance and virulence in subcutaneous infection models [89,90]. *S. aureus* also resists oxidative stress through superoxide dismutases, as confirmed by diminished in vivo survival of mutants lacking these enzymes [91].

SECRETED TOXINS

Numerous toxins secreted by *S. aureus* possess cytolytic activity against host cells and can facilitate tissue spread; promote inflammatory responses; and, especially when the target is a phagocytic cell, promote bacterial innate immune evasion. Perhaps the best-studied toxin is *S. aureus* α-toxin (also referred to as α-hemolysin), which forms heptamers in the membranes of various cell types, creating large pores [92,93]. Pore formation induced by *S. aureus* α-toxin is associated with release of nitric oxide from endothelial cells and stimulation of apoptosis in lymphocytes [94,95]. *S. aureus* production of α-toxin may also promote escape from the phagolysosome after macrophage engulfment [96]. MRSA production of α-toxin is essential for virulence of the pathogen in the mouse model of pneumonia [97]. The level of α-toxin expression by differing *S. aureus* strains directly correlates with their virulence. Immunization with an inactivated form of α-toxin, which cannot form pores, generates antigen-specific IgG responses and provides protection against MRSA pneumonia [98].

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 hypo-osmotic cell lysis. These include γ-hemolysin and the bacteriophage encoded Panton-Valentine leukocidin (PVL) [99]. PVL has gained notoriety because of its strong epidemiologic association with severe cases of CA-MRSA infections [100]. The true contribution of the PVL toxin to *S. aureus* virulence is uncertain. Phage transduction of PVL into a previously naïve *S. aureus* background was reported to increase virulence in a murine necrotizing pneumonia model [101], but an inadvertent mutation in the *agr* regulatory locus of the test strain probably led to spurious interpretations of the PVL linkage to disease pathogenesis [102]. 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 or virulence in murine skin abscess and systemic infection models [103], but did contribute to proinflammatory cytokine release and muscle necrosis at higher inocula and in certain mouse genetic backgrounds [104]. PVL is much more active against human neutrophils than murine neutrophils, explaining sone of the limitations of this animal species as a model for analysis of the virulence functions of the cytotoxin.

Other toxins secreted by *S. aureus* include β-hemolysin, a sphingomyelinase enzyme [105]. Through targeted mutagenesis, β-hemolysin more recently was 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 [106]. Phenol-soluble modulins are a novel family of small, amphipathic, α-helical cytolytic peptides with in vitro and in vivo leukocidal and proinflammatory activities [107]. Phenol-soluble modulins are produced at high levels by CA-MRSA compared with HA-MRSA and contribute to virulence in necrotizing skin and bacteremia mouse models of infection [108].

S. aureus elaborates numerous 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 β-chain of the T-cell receptor, leading to polyclonal T-cell activation and potentially staphylococcal TSS [109]. Twenty distinct *S. aureus* superantigens are known, prominently including TSS toxin-1 and staphylococcal enterotoxins A through E and G through J. The genes encoding the *S. aureus* superantigens are present on accessory genetic elements such as prophages, transposons, plasmids, and chromosomal pathogenicity islands. The contribution of the superantigens to the severe disease manifestations of *S. aureus* are well shown, but the potential evolutionary advantage of superantigen production to the pathogen is unclear. One possible advantage of T-cell activation at the site of infection might be dysregulated cytokine expression patterns that suppress effective local inflammatory responses [109].

Certain strains of *S. aureus* express the exfoliative (epidermolytic) toxins ETA, ETB, ETC, or ETD. These toxins have 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, a desmosomal intercellular adhesion molecule, leading to the exfoliative phenotype of scalded skin syndrome and bullous impetigo [110,111].

QUORUM SENSING AND REGULATION OF VIRULENCE FACTOR EXPRESSION

S. aureus seems to impose tight regulation on the differential expression of specific sets of virulence determinants at different stages of growth or the pathogenic process. Cell wall–associated adhesive factors that facilitate the initial stages of infection are selectively produced during the exponential phase of in vitro growth [112]. 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 [112]. These processes are under the cell density (quorum sensing)–dependent control of the accessory gene regulator (*agr*) locus [113,114]. Similar to other bacterial quorum sensing systems, *agr* encodes an autoactivating peptide (AIP) that is the inducing ligand for a signal receptor (AgrC), the *agr* signal receptor. The unique effector of global gene regulation in the *agr* system is the regulatory RNA molecule, RNAIII [114]. *agr* mutants show decreased virulence in murine infection models [115].

VIRULENCE MECHANISMS OF COAGULASE-NEGATIVE STAPHYLOCOCCI

Until more 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 [116]. 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 [117]. 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 [118]. In addition, plastic objects in the human body soon become coated with host extracellular matrix proteins [119], such that CoNS can colonize the devices either by directly attaching 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 [120]; however, no linkage between surface hydrophobicity and clinical infectivity has been established [117].

Transposon mutagenesis identified AltE, a putative CoNS autolysin protein, as promoting adherence to plastic surfaces [121]; in a rat model of catheter-associated infection, *S. epidermidis* AltE mutant shows diminished pathogenicity [122]. 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 [123].

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

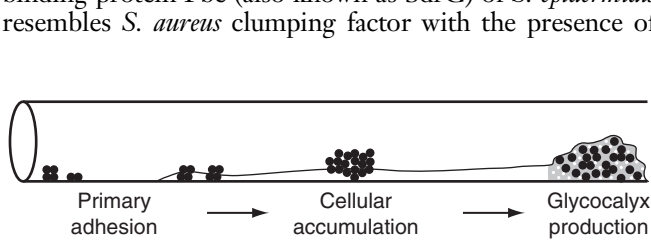


FIGURE 14–3 Schematic model of phases involved in *Staphylococcus epidermidis* biofilm formation.

multiple serine/aspartic repeat domains and a capacity to bind to the β-chain of the host matrix protein [124–126]. *S. epidermidis* cell wall teichoic acid enhances overall adherence to fibronectin, perhaps serving as a bridging molecule between bacterial MSCRAMMs and fibronectin-coated surfaces [127]. Phage-display technology was used to identify *S. epidermidis* protein, EmbP, capable of mediating binding to fibronectin, whereas *S. epidermidis* lipase enzyme, GehD, seems to promote collagen attachment [128]. Finally, the above-mentioned AltE also contains a domain with vitronectin-binding capacity that may contribute to its virulence phenotype in the rat model [121].

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 more than 80% of CoNS isolates from infants with invasive disease [129,130]. 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, PIA [131,132]. PIA is an unbranched β-1, 6-linked *N*-acetylglucosaminic acid polymer, produced by the enzymes of the four-gene *ica* operon [133]. An *ica* knockout mutant shows reduced virulence in a rat model of catheter infection [122], 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 [134]. Expression of 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 [135]. A 140-kDa CoNS extracellular protein known as accumulation-associated protein apparently cooperates with PIA in promoting biofilm growth [136].

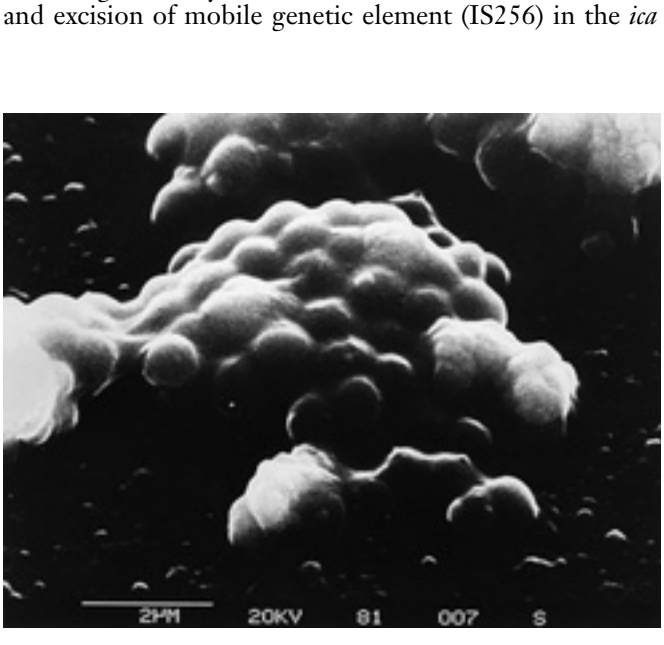


FIGURE 14–4 Scanning electron micrograph showing the presence of a *Staphylococcus epidermidis* biofilm on an explanted intravascular catheter. Biofilm is characterized by multilayered cell clusters embedded in an extracellular polysaccharide. *(From von Eiff C, Peters G, Heilmann C. Pathogenesis of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

CoNS biofilm provides a nonspecific physical barrier to cellular and humoral defense mechanisms [132,137]. The formation of CoNS biofilms depends on the regulatory control exerted by a homologue of the *S. aureus agr* locus [138]. *S. epidermidis* organisms embedded within biofilms bind less complement C3b and IgG and are less susceptible to neutrophil killing [139]. CoNS biofilm–associated polysaccharide also is capable of inhibiting the antimicrobial action of vancomycin and teicoplanin [140]. In the clinical setting, formation of biofilms on the catheter surface has been shown to make eradication of CoNS infection more problematic [141,142].

S. epidermidis expresses a 27-kDa 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 [143]. *S. epidermidis* also expresses a group of secreted amphiphilic peptides called phenol-soluble modulins that have neutrophil chemotactic ability and generate other proinflammatory effects including activating neutrophil oxidative burst and degranulation [144].

ROLE OF THE 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. Localized disease occurs when colonizing organisms are implanted into deeper tissues, often related to a break in skin or mucous membrane integrity and sometimes during placement of a foreign body.

As with other pathogenic bacteria, the presence of intact neutrophil phagocytic function is probably the most important factor involved in controlling replication and spread of staphylococci [145]. The ability of the newborn's bone marrow to respond to infection with rapidly enhanced production and maturation of neutrophil precursors is limited compared with adults [146]. Neutrophils from newborns have relatively diminished motility toward chemoattractants compared with cells from older children and adults [147]; this is partly the result of diminished chemotactic factors such as the complement-derived C5a and the CXC chemokine interleukin (IL)-8 [148,149]. Neutrophils from young infants also exhibit decreased diapedesis across endothelium, possibly because of impaired capacity to upregulate endothelial cell expression of the CR3 receptor [150]. Beyond decreases in neutrophil number, chemotaxis, and transepithelial migration, the capacity for neutrophil adherence and phagocytosis is reduced in neonates, largely

owing to deficiencies in opsonins, including complement and specific antibody [145].

Phagocytic killing seems to be intact in normal newborns, but may be compromised in stressed infants, at least in part because of reduced production of reactive oxygen species [151,152]. The multifaceted antioxidant capacities of *S. aureus*, including catalase and the carotenoid staphyloxanthin pigment, likely support its prominent role as an opportunistic pathogen in stressed infants and in patients with chronic granulomatous disease, where defects in reduced nicotinamide adenine dinucleotide phosphate oxidase lead to marginal oxidative burst function. Chronic granulomatous disease may occasionally be present with *S. aureus*, *Serratia*, or *Aspergillus* infection in the neonatal period [153].

Specific antibody is less important than complement in opsonization of *S. aureus* and plays a limited role in defense against neonatal staphylococcal disease [154]. In general, there is no correlation between antibody titers against *S. aureus* and the likelihood of asymptomatic carriage versus clinical disease [155,156]. Consistent with this information, an attempt to protect the newborn from staphylococcal disease by immunizing the mother near term was unsuccessful [157].

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 [158]. T cells are centrally involved, however, in the immune response to several *S. aureus* toxins, including TSST-1, the staphylococcal enterotoxins, and the staphylococcal exfoliative toxins (ETA, ETB, ETC, and ETD), and in associated pathogenesis. The consequence of this dysregulated T-cell overactivation is proliferation of a large proportion of T cells and release of numerous cytokines, including tumor necrosis factor (TNF)-α, IL-1, and interferon-γ [159]. 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 numerous 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 wide variety of clinical syndromes in the newborn infant, including syndromes with high mortality rates, as was reviewed almost 50 years ago [160]. The clinical manifestations of staphylococcal infection are most prominently a function of two factors: the gestational age of the infant, with extremely low birth weight infants at highest risk of infection and subsequent complications, and the strain of *Staphylococcus* causing the infection, with CoNS generally causing more mild infection compared with *S. aureus*, particularly relevant to more recent CA-MRSA. As noted earlier, staphylococci are armed with an impressive array of virulence factors. They may merely colonize skin or 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 newborns, causing frequent but relatively mild infections in hospitalized premature infants. In contrast, coagulase-positive strains (*S. aureus*) are more commonly associated with clinically aggressive, invasive infections. The subsequent sections provide a general overview of clinical manifestations and organ-specific manifestations.

BACTEREMIA AND SEPSIS

The most common manifestations of invasive staphylococcal infection are bacteremia and sepsis. Studies describing symptomatic community-acquired and hospital-acquired bacteremia in neonates provide an overall framework in which CoNS and *S. aureus* infection can be defined and include early-onset sepsis and late-onset sepsis syndromes [6,25,161–175].

Early-onset sepsis 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 [176]. 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 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 1500 g. 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 includes mottling, poor capillary refill, and metabolic acidosis. In some infants, lethargy, irritability, or poor suck may also be noted.

The incidence of early-onset sepsis caused by *S. aureus* seems 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, cyanosis, and increased oxygen requirement with respiratory distress. In an ongoing 75-year collection of data from Yale–New Haven Hospital [7], *S. aureus* represented the etiology of early-onset sepsis from 28% (1928-1932) to 3% (1979-1988), with a current rate (1989-2003) of 7%. Mortality from all causes of early-onset sepsis declined from about 90% with the earliest data set to approximately 5% of all newborn infants in 1989-2003.

A report from Finland documented *S. aureus* as an etiology of sepsis from 1976-1980 in 22% of all infants with positive blood cultures [177], with an overall mortality rate

of 31%, although for infants with birth weight 1500 g or less, the mortality rate was 44%. Published data from centers in Australia and New Zealand from 1992-1999 documented *S. aureus* rates that varied considerably by year and by institution, with differences in rates even noted within different hospitals in the same city [163], resulting in an overall rate of 19 cases out of 244,718 births (0.008%). In this report, MRSA accounted for only 8% of cases from 1992-1994, but 34% of cases from 1995-1998. Of 26 cases of *S. aureus* sepsis documented in 1999, none were caused by MRSA. The overall mortality rate from MRSA was 25% compared with 10% for MSSA.

Early-onset sepsis caused by CoNS is reported extremely infrequently, likely because of the noninvasive nature of most strains. These reports may reflect true infection, particularly in very low birth weight infants [167,178], 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.

When considering late-onset neonatal sepsis syndrome, occurring after the 5th day of life in hospitalized infants, *S. aureus* and CoNS are well-documented pathogens. In the NICHD Neonatal Research Network, *S. aureus* was the second most common pathogen to cause late-onset sepsis in very low birth weight (401 to 1500 g) infants [6]. CA-MRSA produces particularly devastating infection, with seven of eight 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 [179]. In a maternity hospital in Houston during the same period, mortality attributable to the invasive *S. aureus* infection was 6%, with late sequelae attributable to infection of 12% [179]. 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 during 1993-2003 in Tel Aviv, Israel, mortality rates were virtually identical, 25% versus 27% [172]. In a larger series of 90 infants from Taiwan with bacteremia caused by MRSA, 75% of infants were premature, 54% of infections were believed to be catheter-related, 21% were associated with skin and soft tissue infections, 17% were associated with pneumonia, 8% were associated with bone and joint infection, 3% were associated with meningitis, and 3% were associated with peritonitis [180]. This rate of metastatic infection attributed to MRSA seems greater than that noted with MSSA and is clearly greater than rates seen with CoNS bacteremia. Of 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) [181]. Although the duration of staphylococcal bacteremia was shorter in neonates with MSSA (1 day versus 4.5 days), the mortality and neurodevelopmental outcomes were statistically similar to infants infected with MRSA.

The largest burden of disease in late-onset sepsis caused by staphylococci is catheter-related CoNS bacteremia in premature infants. In the NICU, CoNS cause 40% to 60% of all bacteremic episodes [6,162,182,183]. Rates of catheter-associated bacteremia have been tracked by the U.S. Centers for Disease Control and Prevention [184] and other collaborative groups, including the Pediatric Prevention Network [161] and the Vermont Oxford National Evidence-Based Quality Improvement Collaborative for Neonatology [185]. 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, Texas, during 2000-2002, 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% [178]. Similar findings were published by investigators in the Neonatal Research Network, sponsored by the NICHD [186], highlighting the burden of disease in very low birth weight 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, Benjamin and colleagues [165] noted that the survival of low birth weight infants ($\leq$1250 g) after a positive blood culture for CoNS was virtually identical (8%) to survival of infants evaluated for sepsis yielding sterile blood cultures, in contrast to much higher rates of mortality with gram-negative organisms or *Candida*. 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 [6]. These findings were also confirmed in very low birth weight infants in Israel, reporting on 3462 episodes of late-onset sepsis, documenting a mortality within 72 hours of CoNS bacteremia of only 1.8% [187]. Other authors have suggested that persisting positive blood cultures for CoNS, despite appropriate antibiotic coverage, are associated with an increase in overall complications, with a mortality of 7% [164].

Treatment of catheter-associated CoNS infections is controversial. Karlowicz and colleagues [188] prospectively evaluated treatment with vancomycin versus catheter removal. In neonates treated with vancomycin who experienced clearing of bacteremia within 1 to 2 days, success without catheter removal occurred in 79%, whereas in neonates with persisting bacteremia of 3 to 4 days, the success rate declined to 44%, and in neonates with bacteremia persisting beyond 4 days, none were successfully treated with medical therapy alone, a finding similar to that reported by Benjamin and associates [189] in a retrospective review, in which the rate of metastatic infection increased significantly after four or more positive cultures. Other authors 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 period. 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 a individual case basis, only one of the deaths was possibly attributed to CoNS sepsis [190].

TOXIC SHOCK SYNDROMES

In addition to clinical manifestations related to bacteremia, toxin-mediated clinical disease may occur, including SSSS (see later), TSS [191], and neonatal TSS-like exanthematous disease [192]. 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 [193,194]. TSS has been described in a 4-day-old term infant boy, 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 [191].

A similar disease has been described in Japan, caused by MRSA, producing erythema in association with thrombocytopenia, elevated C-reactive protein, or fever [195]; this presentation has been termed neonatal TSS-like exanthematous disease (Fig. 14–5) [196]. Since the time of the first description, surveys in Japan have shown that 70% of Japanese hospitals have reported a similar illness in neonates [197]. The causative strains all carried the TSST-1 gene and the staphylococcal enterotoxin C gene [192]. The pathophysiology of neonatal TSS-like exanthematous disease begins with colonization with MRSA, a common occurrence among Japanese newborns. Typically, the colonizing strain of MRSA produces TSST-1 [192], and the symptoms of the disease are related to the overactivation of TSST-1–reactive T cells [196]. Neonatal TSS-like exanthematous disease 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 [197].

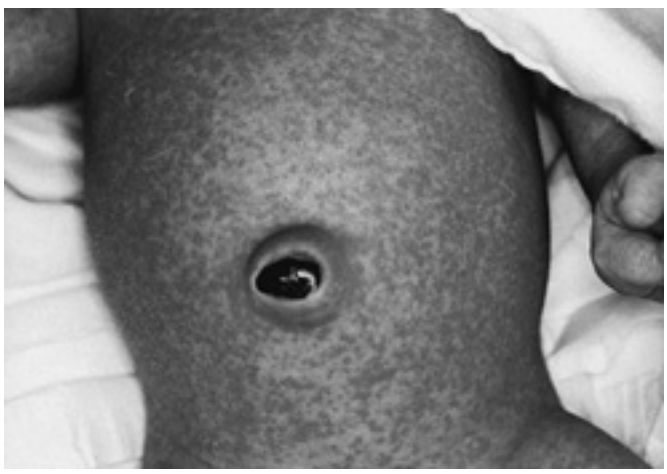


FIGURE 14–5 Typical exanthem in full-term infant with neonatal toxic shock syndrome–like exanthematous disease. *(From Takahashi N, et al. Exanthematous disease induced by toxic shock syndrome toxin 1 in the early neonatal period. Lancet 351:1614-1619, 1998.)*

ENDOCARDITIS

Although infective endocarditis in neonates is rare, autopsy studies from the 1970s revealed unsuspected endocarditis in 0.2% to 3% of neonates who came to autopsy [198,199]. Historically, *S. aureus* has been the predominant bacterial pathogen among neonates with endocarditis [200], but more recent reports indicate that CoNS is now most common [201–204]. 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 [202,204–208]. Endocarditis has also been described in infants infected by CA-MRSA [209].

The signs and symptoms of infective endocarditis in neonates often are nonspecific and similar to signs and symptoms of other conditions such as sepsis or congenital heart disease, including poor feeding, tachycardia, and respiratory distress [200]. Clinical features in general may be unable to distinguish bacteremia with endocarditis from infants with bacteremia without endocarditis [204]. Murmurs can be appreciated in 75% of neonates with endocarditis, with hepatosplenomegaly present in 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 38% [202,207]. The yield of a single blood culture has been reported to be 77% to 97%. When three blood cultures are obtained, the yield approaches 100% [207].

All neonates with *S. aureus* bacteremia should be evaluated by echocardiogram. The thin chest wall of the neonate makes echocardiography a highly sensitive tool for diagnosis of endocarditis in this age group. Limitations of this technique include the inability to detect lesions less than 2 mm in diameter and to differentiate between vegetations and other masses such as thrombi [202]. In all age groups of children, 12% of children with bacteremia with *S. aureus* may have clear evidence of endocarditis; children with underlying congenital heart disease and *S. aureus* bacteremia have a much greater risk of endocarditis compared with children with no cardiac malformations (53% versus 3%) [205]. Mortality in children with *S. aureus* bacteremia and endocarditis has been reported to be 40% [205].

In the Australasian Study Group for Neonatal Infections, bacteremia caused by CoNS in 1281 infants during 1991-2000 was associated with endocarditis in 3 (0.2%); in infants with bacteremia caused by *S. aureus*, endocarditis occurred in 3 of 223 (1.3%) infected with MSSA strains and in 1 of 65 (2%) infected with CA-MRSA strains. Historically, the prognosis for neonates with endocarditis has been grave. Numerous series published in recent years report disease-specific survival rates ranging from 40% to 70% [202,204,207]. Survival of neonates with infective endocarditis is likely to be improved with early diagnosis and aggressive management [200].

PUSTULOSIS, CUTANEOUS ABSCESS, AND 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 (≥36 weeks of gestation). Of infants 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 [40]. Two thirds of all *S. aureus* infections were caused by CA-MRSA, with CA-MRSA and MSSA manifesting with skin or invasive infection in roughly equal percentages; the proportion of infections caused by CA-MRSA increased over the period of observation from 2001-2006 [210,211]. A similar experience was reported from Chicago, in which 11 infants less than 1 month of age 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 [212]. Similar clusters of skin-only pustules and vesicles have been reported from other centers [213].

Evaluation of newborn infants discharged from the hospital, but readmitted within 30 days of age, provided a different profile of clinical disease caused by staphylococci [210]. Infants infected with MRSA presented at 7 to 12 days of age, in contrast to infants infected with MSSA, whose presentations occurred evenly spaced over the 1st month of life. Most 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, 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 III (40-bed) NICU and cultured weekly from the nose and inguinal areas to assess ongoing colonization status showed that of 152 infants known to be colonized over the study period 2002-2004, 6 (3.9%) developed MRSA sepsis, 3 (2%) developed conjunctivitis, 2 (1.3%) developed chest tube site wound infections, and 2 (1.3%) developed cellulitis [214].

ADENITIS AND PAROTITIS

S. aureus cervical adenitis can be another manifestation of nursery colonization in newborns. 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% [215,216]. 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 days and 72 days. Because of the delay in onset of disease, confirmation of a nursery as the source of the infection may be difficult and necessitates careful epidemiologic investigation. Neonatal suppurative parotitis is an uncommon infection among newborns, occurring with an incidence of 13.8 per 10,000 admissions [217]. Premature neonates and boys seem to be at highest risk for suppurative parotitis, which is most frequently caused by *S. aureus* [218,219]. Diagnosis of suppurative parotitis relies on the clinical findings of parotid swelling and purulent exudate from Stensen duct on compression of the parotid gland [220].

BREAST INFECTION

A series of 39 neonatal breast abscesses caused by *S. aureus* were reported by Rudoy and Nelson [221] from Dallas, Texas, in 1975. These infants developed infection most commonly during the 2nd 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 may or may not occur. Culture and Gram stain of purulent discharge is diagnostic. Management includes systemic antistaphylococcal antimicrobials and 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 [221]. Antimicrobial therapy should be provided intravenously until a clear and substantial

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 ages 8 and 15 years [221,222]. A series of three female neonates with necrotizing fasciitis as a complication of breast infection and abscess was 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 [223]. Antimicrobial therapy should be provided intravenously until a clear, substantial response can be documented. In locations with a high prevalence of CA-MRSA, therapy should include clindamycin or vancomycin.

FUNISITIS, OMPHALITIS, AND NECROTIZING FASCIITIS

Funisitis, mild inflammation of the umbilical stump with minimal drainage and minimal erythema in the surrounding tissue, is a local, noninvasive entity. Infections of the umbilical stump may become invasive, however, 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

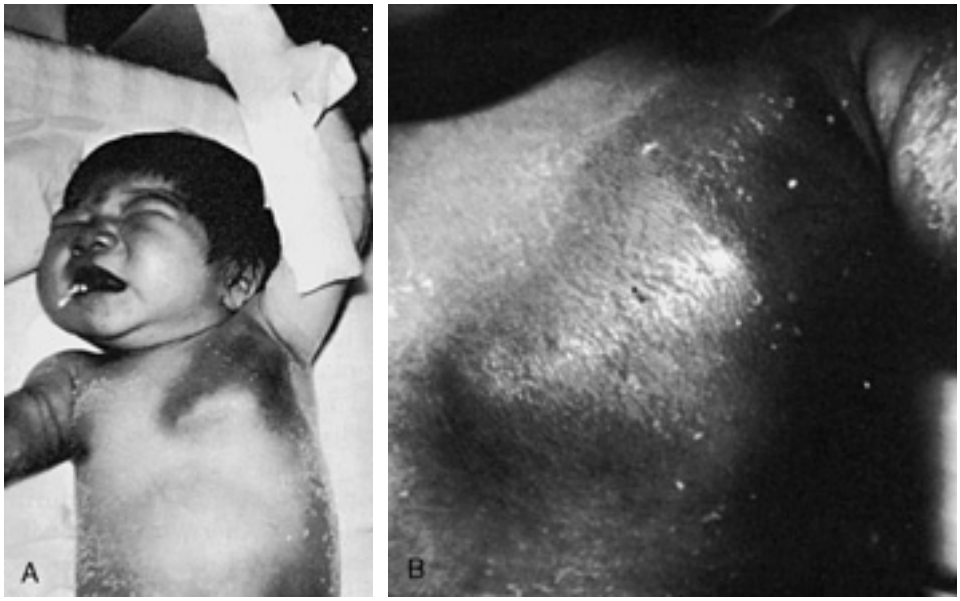


FIGURE 14–6 **A** and **B**, Left breast abscess in a 12-day-old infant. Abscess extends toward the right side of chest and up over the arm. The infant responded well to incision and drainage and antibiotic treatment.

distinct categories: category 1, funisitis and umbilical discharge (shaggy unhealthy umbilical stump, malodorous or purulent discharge); category 2, omphalitis with abdominal wall cellulitis (periumbilical erythema and superficial tenderness in addition to findings in category 1); category 3, omphalitis with systemic sepsis; and category 4, omphalitis with fasciitis (umbilical necrosis with extensive local disease, periumbilical ecchymosis, crepitus bullae, and evidence of involvement of superficial and deep fascia) [224].

Cultures of umbilical tissue in all categories of infection often yield several organisms, including *S. aureus* and CA-MRSA [211,225]. Management of categories 1 through 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. Because *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 seven infants presenting at 4 to 14 days of age with necrotizing fasciitis in Los Angeles, California, four were culture-positive for *S. aureus* in a mixed infection [226]. In Muscat, Oman, 10 of 14 neonates had *S. aureus* cultured from umbilical tissue, including 1 infant positive for MRSA, with 3 of the 10 infants having concurrent staphylococcal bacteremia [227]. Despite aggressive management, the mortality rates of polymicrobial necrotizing fasciitis have been 60% to 70% from sites in the United States [226,227], suggesting that earlier recognition with aggressive surgical management, critical care support, and antimicrobial therapy that includes activity against *S. aureus* or CA-MRSA if appropriate may be necessary to improve outcomes.

Necrotizing fasciitis caused solely by MRSA in the newborn is extremely unusual. The report of the first three cases from the Chang Gung Children's Hospital in Taiwan in 1999 [228] did not include information on the molecular characterization of these strains, raising the possibility that these MRSA strains may not be similar to the currently prevalent USA300 pulsotype, PVL-positive CA-MRSA strains. The clinical course of extensive soft tissue necrosis with relatively mild systemic symptoms, no mortality, and hospital discharge after 3 to 4 weeks of hospitalization is consistent, however, with current reports of CA-MRSA necrotizing fasciitis in 14 adults in Los Angeles and reports of single cases of neonates from San Diego, California, and Chicago, Illinois [229–231].

These neonates present for medical attention at 5 to 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 elevated peripheral white blood count, C-reactive protein, and frequently 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 [232,233]. In addition to broad-spectrum antimicrobials outlined previously and surgical débridement, hyperbaric oxygen treatment has been used, but its role 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 [228,233,234].

STAPHYLOCOCCAL SCALDED SKIN SYNDROME AND BULLOUS IMPETIGO

SSSS has been reported in full-term and premature infants [235–239], with the first reported series of patients in 1878 from Prague by Ritter von Rittershain [240] with clinical infection that is likely to have included patients with SSSS. Clinical characteristics in neonates are similar to characteristics in infants and older children [241] with acute onset of infection associated with macular or generalized erythema 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 show a separation of tissue layers within the epidermis, at the junction of the stratum spinosum and stratum granulosum, owing to 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) [242]. This appearance is distinct from the appearance in toxic epidermal necrolysis, which is characterized by a subepidermal split-thickness 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 shows 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 because there are no substantial 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.

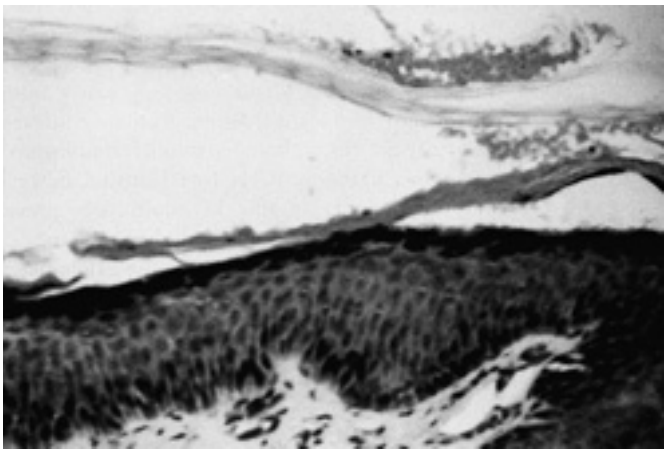


FIGURE 14–7 Photomicrograph of skin biopsy specimen from a patient with staphylococcal scalded skin syndrome, stained with hematoxylin and eosin. Histologic appearance is characterized by epidermal splitting at granular layer of the epidermis. (Magnification approximately 200×.) *(From Hardwick N, Parry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

Localized staphylococcal infection complicating SSSS may also occur with the presence of wound infections, cutaneous abscesses, or conjunctivitis [237,239]. Bacteremia is rare with SSSS, but has been reported [243,244]. Although infection is most commonly described in full-term neonates during the first few months of life, infections in premature infants, including infants with extremely low birth weight, have also been described [235–238]. Scarlatina, as the only clinical manifestation of infection caused by an epidemic strain of SSSS-causing *S. aureus*, has also been observed [239].

Congenital SSSS infection, acquired before delivery as a function of maternal amnionitis, has also been reported in term [244,245] and preterm [246] 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 [239,247].

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 [160,248,249].

In the current era, staphylococcal pulmonary infections produce many different clinical syndromes, depending on the pathogen and presence or absence of underlying lung disease and other comorbidities. The severity of infection caused by CoNS, as with all staphylococcal site-specific infections, is less than that caused by MSSA or CA-MRSA. 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 large series of neonatal sepsis and 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 [6,161–163,175]. The infection is often hospital-acquired in a neonate with underlying lung disease, most commonly chronic lung disease (bronchopulmonary dysplasia), especially in infants receiving concurrent mechanical ventilation.

An early study of staphylococcal pneumonia in the first month of life was reported from New Zealand in 1956 during an epidemic that primarily caused cutaneous infection. The eight infants who died of pneumonia in this epidemic presented at 2 to 3 weeks of age with irritability and poor feeding noted for a few days, followed by dyspnea, cough, and fever [248]. Death occurred in these infants 1 to 5 days

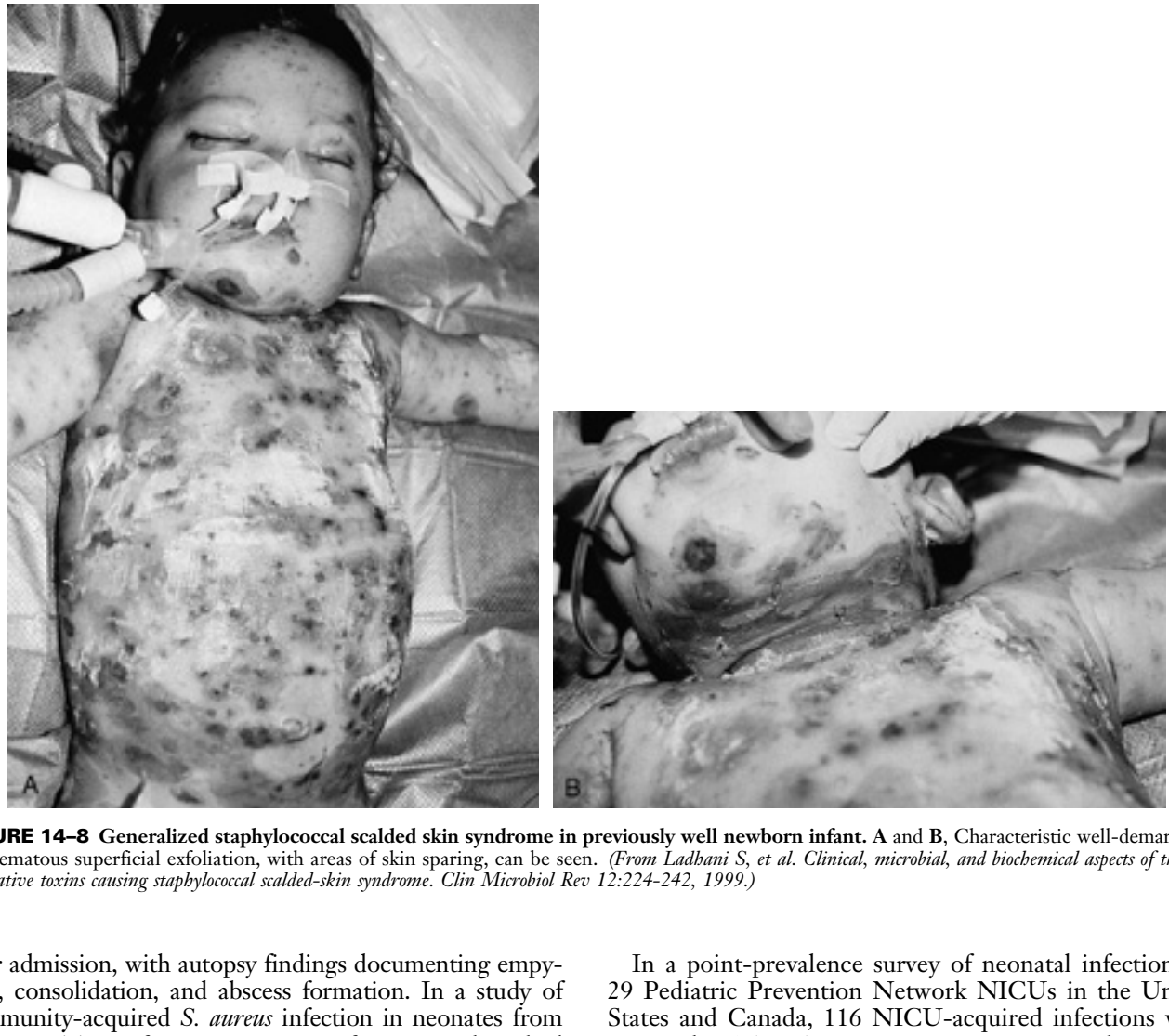


FIGURE 14–8 Generalized staphylococcal scalded skin syndrome in previously well newborn infant. A and **B**, Characteristic well-demarcated erythematous superficial exfoliation, with areas of skin sparing, can be seen. *(From Ladhani S, et al. Clinical, microbial, and biochemical aspects of the exfoliative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

after admission, with autopsy findings documenting empyema, consolidation, and abscess formation. In a study of community-acquired *S. aureus* infection in neonates from Houston, Texas, from 2001-2005, 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 and 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 [25,168,250]. In hospitalized neonates with bacteremia with CA-MRSA in Houston, two of eight had lung involvement: a 24-week gestation 14-day-old infant with empyema, pneumatoceles, and concurrent endocarditis and a 28-week gestation infant with multiple comorbidities including bronchopulmonary dysplasia with infection acquired at 411 days of age, characterized by lung abscess. Both infants died. At present, these cases are uncommon, but they seem to be increasing. The striking severity of CA-MRSA pulmonary disease in the neonate with an extremely high mortality rate despite adequate antimicrobial therapy and supportive care is of great concern. Accurate data on the population-based rates of *S. aureus* pneumonia in neonates are currently unavailable.

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: 15 (13%) were respiratory associated, virtually all in infants with birth weights of 501 to 1500 g; only 2 were associated with CoNS, and only 1 was associated with *S. aureus* [161]. In a review of invasive staphylococcal infections of hospitalized neonates admitted to level II or level III nurseries in Houston, 3 of 41 infants with *S. aureus* infection and 14 of 108 with CoNS infection were documented to have pneumonia. 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 neonates with CoNS. Similarly, bronchopulmonary dysplasia was documented in 65% of *S. aureus*–infected infants and 75% of infants infected by CoNS. In this series, no infant with *S. aureus* pneumonia was documented to develop pneumatoceles or empyemas in the course of infection [178]. The Australasian Study Group for Neonatal Infections collected data on infants with documented bacteremia, occurring at 48 hours to 30 days of age. Of 1281 episodes of CoNS bacteremia, only 6 (0.5%) were documented to have pneumonia [162], 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 [163].

MENINGITIS

Meningitis is infrequently encountered in neonates with *S. aureus* bacteremia [178,210,211], 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 were noted to have meningitis [180]. 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 with MRSA bacteremia (5%) [162]. In most reports in which cerebrospinal fluid white blood cell counts are provided, they are often less than 200 cells/mm^3, however, suggesting that these infants with a cerebrospinal fluid pleocytosis and negative cerebrospinal fluid cultures may not have true staphylococcal bacterial meningitis. Virtually no infant from any series had a positive cerebrospinal fluid culture for *S. aureus*, including 12 infants with bacteremia with a documented pleocytosis from Texas Children's Hospital [211]. It is possible that the pleocytosis represents the entry of staphylococcal cell wall components or inflammatory mediators into cerebrospinal fluid 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 or included in the reports.

In a report from the Australasia Study Group data, of 1281 episodes of CoNS sepsis, 5 (0.4%) were reported to be associated with meningitis [162]. An additional two cases were reported in premature infants from another institution, born at 24 weeks and 25 weeks of gestation, one with a grade IV intraventricular hemorrhage, and developing symptomatic disease at 20 days and 18 days of age. Multiple cultures in both infants confirmed infection caused by *S. epidermidis* in one infant and *S. capitis* and *S. warneri* in the other infant [251].

BRAIN ABSCESS

Brain abscesses caused by *S. aureus* have been described in neonates, most often as a complication of sepsis [252–254]. Clinical presentation includes nonspecific symptoms of systemic infection and a bulging fontanelle; focal neurologic deficits may not occur. The key to diagnosis includes ultrasound or computed tomography (CT) of the head with administration of an intravenous contrast agent and, if not contraindicated, an evaluation of lumbar cerebrospinal fluid. Surgical drainage of the abscess is usually done followed by prolonged antistaphylococcal therapy. Long-term outcome has included neurologic deficits of varying degrees.

Neonates with intraventricular hemorrhage, acute hydrocephalus, congenital malformation, or central nervous system parenchymal injury often require management of increased intracranial pressure by repeated lumbar puncture or by placement of a shunt originating in the cerebrospinal fluid ventricles [255,256]. CoNS are the most common organisms to infect shunt material, producing mild to moderate inflammation and systemic signs and symptoms of infection [255–259]; occasional infection caused by *S. aureus* has also been reported [256,259]. Shunt removal is the preferred method of treatment because sterilization of in situ shunt material is often quite difficult [257]. Treatment with high-dose systemic antimicrobials active against the isolated pathogens, usually vancomycin, 3 to 10 days after 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 cerebrospinal fluid concentrations cannot be achieved with systemic therapy [257,260,261]. 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 [262]. Although 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.

OSTEOARTICULAR INFECTION

Bone and joint infection has long been known to occur with invasive staphylococcal infection in the neonate, with rates of late sequelae of 50% [263–268]. In contrast to infections in older children, the usual distinction between infection of the bone and infection of the joint in a neonate is not as easily made because of 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 [269,270]. 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 [263,269,271]. Virtually all reported cases have been caused by *S. aureus*, although more recent reports document the occurrence of CA-MRSA as a cause of neonatal osteomyelitis [268,272].

The clinical presentation of neonatal bone and joint infections seems to take three general forms. First, the infection may be secondary to staphylococcal sepsis with bacteremia in which case 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 [163,268]. 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 [267,268,272]. In these infants, the clinical findings may be subtle and include signs of irritability, swelling around the affected bone or joint or both, and occasionally failure to move the limb (pseudoparalysis); fever may or may not be present [268,273,274]. Risk factors for bacteremia in these infants include umbilical artery catheterization and prematurity [266–268,275]. In bacteremic disease, the femur and tibia are the most prominently involved bones, infected in approximately 80% of all cases of osteomyelitis [266–268]. 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 [266,268–270]. Because most 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 [163]. Because of delays in diagnosis of osteomyelitis, the location of the infection that often involves bone on both sides of the physis, and frequent involvement of the adjacent joint, late sequelae are common after bacteremic infection; 50% of infants may be described to have long-term sequelae, including limb shortening and decreased range of motion [265–267].

A third clinical scenario leading to a bone or joint infection may be specifically linked to trauma. Osteomyelitis of the calcaneus has been documented to occur as a complication of heel-stick blood sampling, most often manifesting with focal swelling, erythema, and drainage [276]. More recent reports cite continuing problems secondary to a single heel-stick that is used for metabolic screening in all newborns [277]. Fetal scalp monitoring has been associated with skull osteomyelitis [278]. Pyogenic arthritis of the hip is a reported complication of femoral vein venipuncture [279].

Bone radiographs can show destructive changes in the bone becoming apparent in the 2nd 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. Ultrasound may identify collections of pus that are subperiosteal or in the soft tissues. Although radionuclide bone scanning with technetium-99m 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 soft tissues and bone with a lack of ionizing radiation. MRI may be too sensitive, however, 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 agent provides additional information on inflammation in bones and soft tissues and may be particularly helpful when imaging the spine to detect vertebral osteomyelitis or diskitis. CT of neonatal bones and joints has a more limited role in diagnosis of acute infection.

INFECTIONS OF THE GASTROINTESTINAL TRACT

S. aureus is a common colonizer of the gastrointestinal tract of newborns, present in 93% of asymptomatic infants [280]. The prevalence of colonization is not surprising, considering that numerous *S. aureus* organisms can be recovered from samples of breast milk expressed from normal breasts of lactating and nonlactating women [281].

Infections of the gastrointestinal tract can be caused by one of the enterotoxins produced by *S. aureus* or can be a manifestation of invasion of the mucosa resulting in enterocolitis [282]. Although *S. aureus* has been described to colonize the gastrointestinal tract in the absence of clinical disease [280], 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* [283]. Clinical presentation includes signs and symptoms of generalized sepsis in association with frequent, blood-tinged, thin, mucus-containing diarrheal stools. A report of neonatal staphylococcal enterocolitis caused by MRSA described a need for therapy with intravenous and oral vancomycin to establish a microbiologic cure for the systemic infection and colonization; the infant ultimately developed colonic stricture as a late complication of infection [284]. Delta toxin–producing-CoNS [285] and MRSA [286] have not been confirmed to have a major role in the pathogenesis of neonatal necrotizing enterocolitis, although they may have a supporting role in the disease process in some infants.

DIAGNOSIS

In the previously cited reports on clinical manifestations of staphylococcal infection, diagnosis has most often been made by direct culture of the infected tissues or abscesses if the disease is focal or by cultures of blood, urine, or cerebrospinal fluid for diagnosis of sepsis and bacteremia, pyelonephritis, or meningitis and shunt infection. Organism identification and susceptibility testing are essential in understanding the organism-specific severity of disease and 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 total white blood count, immature neutrophil (band-form) count, mature-to-immature white blood cell ratio, C-reactive protein, procalcitonin, cytokines (IL-6, IL-8, IL-10, TNF-α) [287–291], and chemokines [292,293] (interferon-γ-inducible protein-10, monocyte chemoattractant protein-1, RANTES, epithelial neutrophil activating peptide-78), is beyond the scope of this chapter. The sensitivity, specificity, and positive predictive values vary with the investigating institution and the population of neonates studied; some laboratory test results increase 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 [293,294]. At the present time, C-reactive protein and procalcitonin seem 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 [295–297]. No test has the ability to identify all infected infants, with decisions on further investigation and empirical antimicrobial therapy still requiring clinical judgment. With more premature and younger infants, the interval is greater from the time of infection to the time of

a positive nonspecific test for inflammation. MSSA and MRSA *S. aureus* seem to generate far more vigorous responses than CoNS. 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-rRNA 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 [98]. Emerging non–culture-based diagnostic methodologies for neonatal infection are evaluated in detail in Chapter 36.

ANTIBIOTIC TREATMENT

See also Chapter 37.

GENERAL PRINCIPLES

Optimal treatment for staphylococcal infections in neonates is designed to achieve an appropriate antimicrobial exposure at the site of infection and 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. The choice of empirical therapy, before susceptibility test results are known, depends on the local antibiotic resistance patterns for coagulase-positive and coagulase-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 β-lactamase–stable penicillins. Culture and susceptibility information has a direct impact on selection of definitive therapy, allowing the use of the most narrow-spectrum, least toxic antimicrobial regimens.

Although β-lactam agents are preferred for treatment of infections with MSSA 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. 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 unavailable. Extrapolation from other pediatric and adult data is necessary, with cautions for the neonate on outcomes at dosages suggested and on the safety of these antimicrobials.

For antimicrobial therapy of *S. aureus* infections, infections should be separated into MSSA and MRSA. Among MRSA, further differentiation should be made between the more antibiotic-resistant hospital-acquired strains (HA-MRSA) and community-acquired strains (CA-MRSA). No MRSA strains can be killed by penicillin or ampicillin, β-lactamase–stable antistaphylococcal penicillins (methicillin, nafcillin, oxacillin, dicloxacillin), currently available cephalosporins (cephalexin, cephalothin, cefazolin, cefuroxime, cefotaxime, ceftriaxone), or carbapenems (meropenem, imipenem, ertapenem, doripenem). 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, or nafcillin) is preferred. In some cases of mild skin infection, topical antibiotic therapy with mupirocin may suffice. For mild to moderate infections in areas where CA-MRSA occurs at substantial rates (≥5% to 10%), 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 and Erythromycin" subsequently). 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 may be considered in newborns who have responded well initially to intravenous therapy. Trimethoprim-sulfamethoxazole 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 newborns. 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. With data suggesting that the most common pathogen responsible for bloodstream infections in late-onset sepsis in hospitalized neonates is CoNS, most often resistant to β-lactam antibiotics, vancomycin is likely to provide effective therapy. For situations in which cultures show MSSA or methicillin-susceptible or penicillin-susceptible CoNS, it is imperative that therapy be switched back to traditional β-lactam antibiotics to minimize antibiotic pressure on staphylococcal species from vancomycin or clindamycin, to delay the emergence of resistance to these antibiotics. β-lactam antibiotics are generally 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. Within every population of *S. aureus*, a very low frequency of organisms with intermediate resistance to vancomycin exists, however, and these organisms may become selected out in infants with prolonged exposure to vancomycin. 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 designed to achieve an area under the curve-to-minimal inhibitory concentration ratio of approximately 250 and is associated with microbiologic cure in experimental in vitro and in vivo animal models and in retrospective analyses of infections in adults [299]. In neonates, many dosing recommendations exist, including intermittent dosing and continuous infusion, primarily based on chronologic and gestational age and based on serum creatinine [300–304]. An initial loading dose of 15 mg/kg is most often recommended, 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. 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 central nervous system infections, primarily ventriculoperitoneal shunt infections caused by CoNS [260,261], although cerebrospinal fluid concentrations may be therapeutic after intravenous administration [301].

CLINDAMYCIN AND ERYTHROMYCIN

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 occur that constitutively produce methylase, providing complete resistance to all macrolides (erythromycin, azithromycin, clarithromycin), clindamycin, and 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 shows 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 a 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, 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 neonates.

LINEZOLID

Of the antibiotics approved during the past decade with activity against MRSA, linezolid is the only one currently approved by the U.S. Food and Drug Administration (FDA) for use in neonates. 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 [305]. 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 coagulase-positive and coagulase-negative staphylococci. Data on pharmacokinetics are available for all pediatric age groups, including premature neonates less than 34 weeks' gestational age.

Linezolid can be administered 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 into tissues. Linezolid is cleared by the kidneys, unchanged and after oxidation of the parent compound. Because oxidation of linezolid does not depend 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 [306,307]. 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*, including MSSA and MRSA strains, and response rates for infections caused by CoNS were also statistically equivalent to vancomycin. Similarly, the rates for clinical and laboratory adverse events were equivalent to adverse events in vancomycin-treated control patients. In neonates and children enrolled in these registration trials, the hematologic toxicity profiles for neutropenia and thrombocytopenia were equivalent to vancomycin. 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 <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. 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.

In studies of cerebrospinal fluid 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 central nervous system infection in a neonate [262], the routine use of linezolid for the treatment of central nervous system 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 remain to be defined. The role of combination therapy using linezolid is also not defined.

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. Pharmacokinetic studies are ongoing in older children, but no data exist for neonates. 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) and for bacteremia and endocarditis. Daptomycin also shows in vitro activity against vancomycin-resistant *S. aureus* and should represent an effective agent if these strains become more widespread. Daptomycin is not indicated for the treatment of pneumonia because surfactant binding to the antibiotic is associated with inactivation. Myopathy is a potential adverse event, which was noted in early phase 1 studies, but with once-daily dosing in adults, no muscle toxicity was documented. Current guidelines suggest monitoring serum creatine phosphokinase concentrations weekly.

QUINUPRISTIN-DALFOPRISTIN

Streptogramins are antibiotic derivatives of natural products of *Streptomyces pristinaespiralis*. Two streptogramins, quinupristin and dalfopristin, 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 for adults as Synercid, for the treatment of vancomycin-resistant *Enterococcus faecium* infections and for the treatment of skin and skin structure infections caused by *S. aureus* (only MSSA strains were isolated from study patients). In vitro, quinupristin-dalfopristin is also active against MRSA and vancomycin-resistant *S. aureus*, although no clinical data are available for treatment of these infections. Quinupristin-dalfopristin 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. 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 data, in vitro data, and data from CoNS infections and in the absence of human data for *S. aureus* [308]. For MSSA endocarditis, combination therapy with a β-lactam penicillin (oxacillin or nafcillin) and 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 [308]. A report on vancomycin plus rifampin combination therapy of persisting CoNS bacteremia after removal of a central catheter provides some support to this approach [309].

A Cochrane review of intravenous immunoglobulin therapy of suspected or documented neonatal sepsis evaluated nine clinical trials. Although substantial heterogeneity existed across studies in immunoglobulin preparations, dosing regimens, and populations studied, no substantial benefit was derived from treatment, particularly with respect to mortality in infants with either documented suspected infection or subsequently proven infection [310].

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 [189]. For infants with CoNS bacteremia, successful treatment of bacteremia may be possible with the central venous catheter in situ [189]. If bacteremia persists for longer than 4 days, the chance for subsequent clearance is reduced [188], however, and the risk of end-organ damage may be increased [164,189]. 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. Prompt removal of an indwelling central venous catheter should be considered in infants with central nervous system hardware [188].

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 occurrence of infections 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 fomites; overcrowding of infants in the 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 to be effective in helping to curtail the outbreak, even in the case of MRSA [311].

A primary determinant of infant colonization is nursing care. Maintaining an appropriate nurse-to-infant ratio is an important factor in reducing disease when a disease-associated *S. aureus* strain gains entrance to a nursery, especially in the NICU [312]. In addition, various preventive maneuvers are directed at persons with direct infant contact, including frequent mask, gown, and glove changes before handling of infants [313,314]; application of antimicrobial or antiseptic ointment or spray [315,316]; and elimination of carriers from the nursery area [317,318]. In some situations, control of an epidemic requires removal of the nurse carrier from the nursery [319].

Currently, the U.S. Centers for Disease Control and Prevention recommends contact isolation for patients colonized or infected with MRSA [320]. This practice was shown to reduce nosocomial transmission of MRSA by 16-fold during an outbreak of MRSA in an NICU [321]. Several more 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 [322–325].

In the early 1960s, attempts were made to stop virulent *S. aureus* epidemics in 10 NICUs throughout the United States using the technique of bacterial interference [326,327]. This technique involved deliberate implantation of *S. aureus* 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 [328], 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 [329] 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 [330,331]. Hands must be cleaned before and after patient contact or contact with equipment that is used for patient care. Hands also should be cleaned after glove removal. Proper hand hygiene involves applying alcohol-based waterless rubs if hands are not soiled [332] or washing the hands for at least 10 to 15 seconds with either chlorhexidine gluconate or triclosan hand-washing agents [333].

With the increase 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 intra-arterial catheters may decrease the risk of catheter-related infections [334]. In patients who require intravenous access for prolonged periods, percutaneous placement of a small-diameter Silastic catheter is preferred when possible. In one study, these catheters were maintained for 80 days, with an infection rate of less than 10% in infants weighing less than 1500 g [335].

ANTIBIOTIC PROPHYLAXIS

Investigational therapies to reduce neonatal bacteremia caused by staphylococci have been directed at the use of antibiotic prophylaxis and antibiotic-impregnated devices. Given the large burden of CoNS catheter infections in premature infants, investigations of prophylactic antibiotics to prevent infection were undertaken by many institutions, as reviewed more recently [336–340]. 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 [339]; the administration of low-dose vancomycin at 5 mg/kg twice daily [338]; 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.

IMMUNOPROPHYLAXIS

Studies evaluating the effectiveness of immunoglobulin preparations generally have not documented convincing, substantial benefits for the populations of premature infants studied [310]. These studies may reflect the lack of effectiveness of a specific biologic preparation, however, or suggest that particular subpopulations may benefit more from treatment than others, rather than proving that immunoglobulins have no potential role in prophylaxis or treatment. Other polyclonal antibody

approaches to prophylaxis in premature infants have used high-titer anti–*S. aureus* immunoglobulin (Altastaph), prepared from adult volunteers immunized with a staphylococcal vaccine. Pharmacokinetic, safety, and clinical outcome data in neonates randomly assigned to receive either immunoglobulin or placebo did not show benefit in early, limited trials [341].

Studies of monoclonal antibodies directed against specific staphylococcal epitopes are ongoing. A randomized, placebo-controlled trial was 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, ClfA, and Ser-Asp dipeptide repeat G (INH-A00021, Veronate). No benefit to prophylaxis was noted in the recipients of this staphylococcus-specific immunoglobulin [342,343]. An anti-staphylococcal monoclonal antibody, BSYX-A110, 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 [344]. The efficacy of the antibody in preventing CoNS infections and related morbidity and mortality remains to be established.

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 [345]. A randomized study of bovine lactoferrin supplementation in very low birth weight premature infants showed a promising reduction in the rate of late-onset sepsis in the treatment group (risk ratio 0.34, 95% confidence interval 0.17 to 0.70) [346].

CONCLUSION

Staphylococcal infections result in significant morbidity and mortality in neonates. 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 emergence of CA-MRSA with exceptionally high mortality rates have created an unprecedented need to understand the biology and mechanisms of virulence of staphylococci. With this understanding, we can generate improved approaches to prevent and treat infections. A profound need exists to develop more safe and effective antimicrobials and immunotherapies to mitigate the substantial morbidity and mortality caused by these pathogens.

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