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Osteomyelitis: Clinical Overview and Mechanisms of Infection Persistence

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Abstract

Osteomyelitis is a progressive infection of bone, that results in inflammatory destruction of the bone, bone necrosis, and new bone formation and may progress to a chronic and persistent state. The major categories of osteomyelitis are based on the source of infection (hematogenous or secondary to a contiguous focus of infection) and whether vascular insufficiency (either local or systemic) exists. While large-organism inoculation and/or host compromise can predispose patients to the development of osteomyelitis, the virulence of the infecting pathogen also has a significant role. One species in particular, *Staphylococcus aureus*, is able to cause an acute bone infection even with a low inoculum in a healthy host. In addition, through the timed expression of its arsenal of virulence factors and aided by its ability to develop antibiotic resistance rapidly, *S. aureus* progresses to a chronic, biofilm-mediated infection. Once a chronic infection develops, bacterial clearance cannot be attained by the host immune system or antimicrobial therapy. At this point, surgical removal of the nidus of infection is usually necessary for complete infection resolution.

Osteomyelitis, or infection of the bone (1), is a continuing issue facing clinicians, causing thousands of hospital admissions each year in the United States. Bone is relatively resistant to infection. However, osteomyelitis can occur if there is a large-organism inoculation, trauma with resulting bone damage occurs, foreign bodies (such as prosthetic implants) are present, or a particularly virulent bacterial species (e.g., *Staphylococcus aureus*) gains access to the bone. Therefore, the occurrence, type, severity, and clinical prognosis of osteomyelitis depend on the interplay of a triad of factors,

including the characteristics and virulence of the infecting pathogen, the properties of the host, and the source of infection.

Once the bone is colonized and an active, acute infection occurs, there are three potential results. The infection may resolve, become a quiescent and enduring infection, or become a chronic infection with related progressive bone deterioration and infection extension (2). The pathogenesis of osteomyelitis has been studied clinically, and various types of osteomyelitis can be grouped according to the source of the infection (i.e., hematogenous or contiguous focus) and whether the patient has sufficient local or systemic blood flow (3-5). To understand the molecular mechanisms of microbial persistence, following a clinical overview of osteomyelitis, the chief etiological agent of osteomyelitis, *S. aureus*, will be used to describe how

an acute and treatable infection can readily progress to a chronic, persistent disease.

Hematogenous Osteomyelitis

Hematogenous osteomyelitis results from the spread of bacteria from the bloodstream into the bone and accounts for 20% of osteomyelitis cases. Hematogenous osteomyelitis can be subdivided into primary and secondary categories. Primary hematogenous osteomyelitis is caused by the direct seeding of bone from bacteria in the blood. Although found in the adult population, it is more predominant in infants and children,

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with 85% of cases occurring in patients younger than 17 years of age (3). Recent studies have documented a decline in hematogenous osteomyelitis, especially in children, possibly due to the large decrease in osteomyelitis caused by *Haemophilus influenzae* resulting from the modern vaccination program (6,7). Hematogenous osteomyelitis in adults is more commonly caused secondarily from a distal site of infection. These infections can also stem from the reactivation of a quiescent focus of osteomyelitis that arose in infancy or childhood and “arrested.” The most common site of involvement is the distal part of the tibia, and the lesion is usually single and located near the metaphysis. Two common types of hematogenous osteomyelitis are long-bone and vertebral osteomyelitis. Both are most often caused by a monomicrobial infection, though vertebral osteomyelitis secondary to trauma may be polymicrobial.

In long-bone osteomyelitis, the metaphyses of the long bones (tibia and femur) are most frequently involved (8), and the anatomy of the metaphyseal area seems to explain this localization (9). The nutrient artery ends in the metaphyses, as tight capillaries close to the growth plate make abrupt loops. Blood from these capillaries then enters a system of large venous sinusoids, where the blood flow becomes slow. These capillary loops are fundamentally the “end-artery” branches of the nutrient artery. This structure allows the slowing of blood flow in this area, which permits bacteria to settle and initiate an inflammatory response. The metaphyseal capillaries are also lacking in phagocytic lining cells, and the sinusoidal veins contain functionally inactive phagocytic cells (10), leading to a decreased immune response that subsequently leads to further growth of microorganisms.

Infants and children may be predisposed to infection via minor trauma that produces a small hematoma, vascular obstruction, and subsequent bone necrosis that is vulnerable to inoculation from a transient bacteremia (11). Acute infection at first produces a local cellulitis that leads to a breakdown of leukocytes, increased bone pressure, decreased pH, and decreased oxygen tension. The combined effects of these physiologic factors further compromise the medullary circulation and thus increase the spread of infection. Infection may advance laterally through the Haversian and Volkmann canal systems, perforate the bony cortex, and lift the periosteum from the surface of the bone. When this occurs in the presence of medullary extension, the periosteal and endosteal circulations are compromised. Also, capillaries are lost and large segments of cortical and cancellous bone die. In infants, infection may spread to the joint surfaces through the vascularized growth plate (12), but in children older than 1 year, the growth plate lacks capillaries and the infection is confined to the metaphysis and diaphysis. The joint is spared unless the metaphysis is intracapsular. In the long bones of adults, the infection frequently begins in the diaphysis but may extend to involve the entire medullary canal. Extension into the epiphysis and joint space may occur, because the growth plate has disappeared and the medullary areas are adjacent. As the periosteum is tightly fixed to the bone, cortical penetration usually leads to a soft tissue abscess, but subperiosteal abscesses and massive cortical devitalization rarely occur. Eventually, sinus tracts connecting the sequestered nidus of infection to the skin via soft tissue extension may form. The clinical signs resulting from soft tissue extension often dominate the clinical findings at presentation and can lead to inappropriate diagnostic and

therapeutic measures unless one also considers the possibility of underlying bone infection.

In chronic hematogenous osteomyelitis, the existing cortex is usually viable. The involucrum contains the sequestered, necrotic marrow and endosteal bone. Sequestra are often found within the thickened cortex and are bordered by reactive bone and chronic granulations. These sequestered, lamellar fragments are seldom responsible for an acute exacerbation of infection but can support a progressive compromise of ischemic soft tissues that leads to chronic ulceration and drainage.

Contiguous-Focus Osteomyelitis

Although the term “contiguous focus” implies that the infection stems from an adjacent soft tissue infection, chronic contiguous-focus osteomyelitis can also begin as an acute infection, with the organisms being directly inoculated into the bone at the time of trauma. Infection can also be spread by nosocomial contamination during preoperative or intraoperative procedures. Osteomyelitis secondary to contiguous foci of infection accounts for at least half of all cases (3). A recent study has documented a decline in hematogenous osteomyelitis, with a concurrent rise in contiguous disease (6). Contiguous-focus osteomyelitis is biphasic in terms of the ages it affects, with infection occurring in younger individuals as a result of trauma and related surgery and in older adults secondary to surgical procedures and decubitus ulcers.

Patients who commonly suffer from contiguous focus osteomyelitis are those with generalized vascular insufficiency, which makes appropriate therapy and management complex. Most of the patients fitting this description have diabetes mellitus (13) and range from 35 to 70 years of age. The small bones of the feet, talus, calcaneus, distal

fibula, and tibia are often involved in this kind of infection. Often, the infection is initiated by minor trauma to the feet, such as infected nail beds, cellulitis, or trophic skin ulceration.

It can be difficult to diagnose osteomyelitis in patients with compromised vasculature. The patient may present with an ingrown toenail, a perforating foot ulcer, cellulitis, or a deep-space infection. Examination shows decreased dorsal pedis and posterior tibia pulses, poor capillary refill, and decreased sensation, but fever and systemic toxicity are often absent. Concurrent peripheral neuropathy deadens the patient's perception of pain. Although arrest of the infection is the optimal goal, a more feasible objective is to control the infection and maintain the functional integrity of the involved limb. Débridement and ablation are often necessary. Prior to the surgery, the causal host disorders (diabetes, poor nutrition, or peripheral vascular disease) should be treated. The intractable nature of this type of infection frequently leads to persistent bone infections, even after appropriate therapy. Removal of the infected sections of the bone is almost always needed.

Diagnosis and Treatment of Osteomyelitis

Diagnosis of osteomyelitis can be very difficult. There are several imaging methods that allow clinicians to visualize the region of possible infection. Conventional radiography is often used. However, radiographic changes in bone are often difficult to interpret, and it can take at up to 2 weeks following the onset of infection to reach the 30 to 50% loss in bone density that is often required for visualization (14). The earliest signs seen on a radiograph are soft tissue swelling and thickening of the periosteum, and abscess formation. However, these are easily missed. Also, it can be difficult to determine the extent of infection. Sensitivity and specificity are only 70% and 50%, respectively, making this technique somewhat unreliable (15). Ultrasound is another option, but is only able to diagnose soft tissue infection surrounding the bone and thus is not effective in the diagnosis of osteomyelitis. Computed tomography scans can be of more use because of their high level of detail, particularly in

cases of vertebral osteomyelitis. However, because of the problem of scatter when metal implants are present, this method is not always useful in the case of infected implants. It is also a very expensive procedure, which further limits its usefulness. Radionuclide scans are widely used, and they help to identify areas of inflammation better than radiography alone. These methods also have the benefit of being useful for suspected implant infections as well, since there are no issues with scatter. One technique, ^{99m}technetium-^{99m}methylene diphosphonate scintigraphy has proven to be effective and relies on pharmaceutical accumulation at areas of increased blood flow and bone repair. The sensitivity and specificity of osteomyelitis diagnosis for this method range from 69 to 100% and 38 to 94%, respectively. However, any event leading to bone injury leads to a positive scan, causing false diagnoses. False-positive rates from 0% to 64% have been reported (16), and high rates can be attributed to cases of new bone formation, fracture healing, heterotopic ossification, arthritis, and local minor trauma (17). Another radionuclide technique exploits ¹¹¹Indium (¹¹¹In)-labeled white blood cells: patient leukocytes are isolated, labeled with ¹¹¹In, and injected back into the patient. These radiolabeled leukocytes accumulate in regions of acute infection, and thus, it is a sensitive method (except in many cases of chronic osteomyelitis) and the radionuclide technique of choice for diagnosing and localizing osteomyelitis in the limbs (18). While its sensitivity is 86%, its specificity is only 12% (19). However, when combined with bone marrow imaging with ^{99m}Tc sulfur colloid marrow scintigraphy, this method works well in cases of suspected prosthetic implant infection, because leukocyte uptake around prostheses may be caused by surgery. When an accumulation of leukocytes is seen, coupled with noncongruent bone marrow patterns and absent marrow uptake, an infection is likely. ^{99m}Tc hexamethylpropylene amineoxime is also used to overcome the problems with use of ¹¹¹In-labeled white blood cells, such as the 24-h delay required for imaging, high levels of radiation in the spleen, and limited injection dose. The combination of these two scans led to a sensitivity of

100% and a specificity of 94% in one study (19). Magnetic resonance imaging (MRI) is the final imaging technique commonly used. This method allows differentiation between bone and soft tissue infection, and the sensitivity and specificity to detect cases of osteomyelitis are between 68 and 100% and 50 and 100%, respectively (20,21). In cases of vertebral osteomyelitis, MRI is particularly valuable and has been shown in one study to have a sensitivity and specificity of 96% and 92%, respectively (22). However, MRI also has the issue of scatter when metal implants are present.

The gold standard of diagnosis for osteomyelitis is to obtain a biopsy specimen and culture it for the infecting organism (23). Even with the imaging tools described above, a biopsy specimen of the bone is often necessary to determine if osteomyelitis rather than other inflammatory processes is present. Until a positive identification of the etiologic agent is made, the proper therapeutic treatment is delayed.

Once a positive diagnosis of infection has been made, treatment for osteomyelitis consists of antimicrobial therapy, débridement, and follow-up care that includes stabilization of the bone and management of any dead space that remains after débridement (23). Most often, a broad-spectrum antibiotic is given until culture results are obtained to cover the most common pathogens, and then specific therapy is begun once the infecting agent is identified. Antibiotic treatment must be administered for at least 4 to 6 weeks. However, few studies have been completed on treatment of osteomyelitis, so currently, it is not known if there are better means of treatment. For *S. aureus* infections, particularly methicillin-resistant *S. aureus* (MRSA), the antibiotic of choice is vancomycin, with teicoplanin, trimethoprim-sulfamethoxazole, or minocycline plus rifampin as alternatives (23).

Microbial Species Responsible for Osteomyelitis

Hematogenous osteomyelitis is generally monomicrobial in nature (i.e., a single bacterial species is isolated from the infected region). Polymicrobial hematogenous osteomyelitis is rare (3-5). In infants, *S. aureus*, *Streptococcus*

agalactiae, and *Escherichia coli* are the most frequently recovered bone isolates, while in the child, *S. aureus*, *Streptococcus pyogenes*, and *H. influenzae* are the most common organisms isolated. After the age of 4, the incidence of *H. influenzae* osteomyelitis decreases. However, as mentioned above, the overall incidence of *H. influenzae* as a cause of osteomyelitis is decreasing because of the new *H. influenzae* vaccine now given to children (7,24). In adults, *S. aureus* is the most common organism isolated (8). *Staphylococcus* spp. can cause osteomyelitis in immunologically normal children and adults, as well as in immature and immunocompromised individuals. Overall, some other pathogenic microorganisms associated with osteomyelitis are *Enterococcus* spp., *Streptococcus* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Mycobacterium* spp., and anaerobes and fungi (specifically *Candida* spp.). Each of these pathogenic species represents a small minority of infections. In vertebral osteomyelitis, aerobic gram-negative rods are often found, with the urinary tract or intravenous drug use as the source of infection. *P. aeruginosa* and *Serratia marcescens* are found in high incidence in intravenous drug users (25,26).

In contrast to the case in hematogenous osteomyelitis, multiple organisms are usually isolated from the bone in contiguous-focus osteomyelitis. *S. aureus* and coagulase-negative *Staphylococcus* spp. account for 75% of the bacterial isolates (3-5). However, gram-negative bacilli and anaerobic organisms are frequently isolated. A high rate of nasal and skin colonization with *S. aureus*, defects in host immunity, and impaired wound healing all play roles in foot infection. Superficial fungal skin infections, which are common in diabetic patients, may also allow bacteria entry through macerated or broken skin. Multiple organisms are found in patients with osteomyelitis involving the small bones of the feet, including *S. aureus*, coagulase-negative *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., gram-negative bacilli, and anaerobes. Aerobic gram-negative bacilli are usually a part of a mixed infection (13).

One bacterial species, *S. aureus*, is capable of producing a serious acute

infection that readily transitions into a chronic infection regardless of host immune status. Once in the chronic form, surgical intervention is generally required to resolve the infection. *S. aureus* has been studied extensively with regard to its role in osteomyelitis, and it causes the majority of the infectious cases. This bacterial species is discussed here as the "typical" pathogen of bone.

S. aureus

General properties

S. aureus is a gram-positive, facultatively anaerobic coccus that is non-motile and non-sporeforming. Also, it is coagulase-positive, which allows it to cause plasma to clot in vitro and also distinguishes it from other common staphylococci (e.g., *S. epidermidis*) (27). *S. aureus* is a normal commensal of the human nares. Approximately 20% of the population are consistently colonized with this bacterium, while 60% of the population are transient carriers (28). *S. aureus* infection can lead to several diseases, ranging from minor skin infections (e.g., furuncles and boils) and eye infections (e.g., keratitis) to more serious illnesses, including bacteremia, endocarditis, septic arthritis, wound infections, pneumonia, toxic shock syndrome, and osteomyelitis. Incidences of *S. aureus* infection have become more worrisome with the emergence of multiple antibiotic-resistant strains, such as MRSA. Approximately 40 to 60% of all nosocomially acquired *S. aureus* strains are methicillin-resistant, and these strains are now considered endemic in hospitals (29). Until recently, the only drug to which all *S. aureus* strains were susceptible was vancomycin, but strains with reduced susceptibility or resistant to vancomycin are beginning to be isolated as well (30). *S. aureus* can also cause infections when acquired in the community rather than the hospital. Though these infections tend to be skin related, community-acquired strains also acquire methicillin resistance and are of greater concern, not only because they are becoming more virulent (29), but also because these strains can infect hosts outside of the hospital setting who have no predisposing risk factors. *S. aureus* is able to cause acute osteomyelitis. The disease progresses to a chronic, biofilm-mediated infection because of the ability of

S. aureus to rapidly develop antibiotic resistance and the timed expression of its arsenal of virulence factors.

Antibiotic resistance

In the pre-antibiotic era, bacteremia with *S. aureus* resulted in a 90% death rate (31). Though considerably better today, with the ever-increasing level of antibiotic resistance and the evolution of community-acquired resistant strains, the health risk associated with this pathogen is sure to rise.

β -lactam antibiotics, such as methicillin, oxacillin, and penicillin, act on the bacterial cell wall. These antibiotics work by inactivating transpeptidases, so that the peptidoglycan of the cell wall cannot be synthesized. In MRSA, the *mecA* gene, which encodes the penicillin-binding protein PBP2a, is horizontally acquired, and this bacterial product takes over the functions of the inactivated transpeptidases and is naturally resistant to β -lactams (32).

Because β -lactams are ineffective against MRSA, glycopeptides, such as vancomycin and teicoplanin, have become the drugs of choice for treatment (33). However, as mentioned above, *S. aureus* strains with an intermediate susceptibility to glycopeptides are beginning to emerge, as are strains that are resistant to the antibiotic (34). Vancomycin-resistant *S. aureus* strains have gained the *vanA* resistance gene from enterococci that are also resistant to vancomycin (29), while in strains showing reduced susceptibility to vancomycin, the cell wall is thicker than in susceptible strains (34). The rather rapid acquisition of both methicillin and vancomycin resistance underscores the fact that *S. aureus* has been capable of becoming resistant to any drug it has been faced with, making current and future treatment difficult. Antimicrobial resistance, particularly methicillin resistance, usually results in a delay of appropriate and effective antimicrobial therapy. This delay increases the chances for the development of chronic osteomyelitis and prosthetic-implant infection.

Virulence factors

S. aureus has an array of virulence factors that have roles in infection and that may be classified as being responsible for adherence, direct host damage, or immunoavoidance. These factors have specific roles in the colonization

and infection process in bone infections, and their expression is coordinated throughout the various stages of infection.

During early exponential growth, when cell density is low, proteins that promote adherence and colonization (such as fibronectin-binding protein, protein A, staphylokinase, and coagulase) are expressed. When cell growth reaches high densities, the production of the adherence and colonization factors is suppressed, while secreted toxins and enzymes are expressed, such as enterotoxins; epidermolytic (exfoliative) toxin A; alpha, beta, and delta hemolysin; serine protease; nuclease; type 5 capsular polysaccharide; clumping factor; leukocidin; phosphatidyl-specific phospholipase C; fatty acid-modifying enzyme; lipase; hyaluronate lyase (hyaluronidase); and toxic shock syndrome toxin 1. Many of these post-exponential-phase proteins are involved in damaging the host, obtaining nutrients from the host for bacterial growth, and dissemination once the staphylococci have adequately colonized and increased in number to promote an active infection. The expression of most of these staphylococcal products is under partial or complete control of the staphylococcal accessory regulator (*sar*) and the accessory gene regulator (*agr*) quorum-sensing systems. In addition, several environmental signals have been implicated in the *sar/agr*-dependent or independent regulation of virulence factors. These signals include non-maintained pH (35), osmolarity (36,37), glucose level (38), DNA topology (36), NaCl, sucrose level (39), temperature (40), amino acid availability (41), and the presence of O₂ and CO₂. Also, a homologue to the ferric uptake regulator (*fur*) of gram-negative organisms was recently isolated and was found to regulate the *sir* operon (staphylococcal iron regulated) that has been proposed to constitute a siderophore transport system in *S. aureus* (42). Many of the reactions of *S. aureus* to these environmental cues are classed as stress responses, and they are believed to be regulated by sigma factors that control gene expression. While these virulence factors and their regulation enable colonization and the development of an acute infection, the formation of a bacterial biofilm is at the heart of chronic

osteomyelitis.

Biofilms

Staphylococcus spp. can produce a multilayered biofilm embedded within a glycocalyx, or slime layer (43). The glycocalyx develops on devitalized tissue and bone (such as the involucrum) or on medically implanted devices to produce an infection (44). The presence of implants is a predisposing factor in the development of infection, since they are coated in host proteins soon after implantation, and this host protein coating provides an excellent source of attachment for any bacteria remaining after debridement surgery (45). Once attached, the bacteria can form the glycocalyx, which protects them from normal host defenses and systemic antibiotics (46).

Early studies described the solid component of the glycocalyx as primarily composed of teichoic acids (80%) and staphylococcal and host proteins (47). Host-derived proteins, such as fibrin, may be derived from the conversion of fibrinogen by the staphylococcal coagulase-prothrombin complex (48). In later studies, a specific polysaccharide antigen named polysaccharide intercellular antigen (PIA) was isolated. PIA is composed of β -1,6-linked N-acetylglucosamine residues (80 to 85%) and an anionic fraction with a lower content of non-N-acetylated D-glucosaminyl residues that contains phosphate and ester-linked succinate (15 to 20%) (49). PIA is a polymer of approximately 130 residues, but other sizes of this β -1,6-linked N-acetylglucosamine have been identified, termed PNAG-I (the immunogenic 460-kDa compound), -II (100 kDa), and -III (21 kDa) (50). PIA is produced in vitro from UDP-N-acetylglucosamine via products of the intercellular adhesion (*ica*) locus (51). The genes and products of the *ica* locus (*icaR* [regulatory] and *icaADBC* [biosynthetic] genes) have been demonstrated to be necessary for biofilm formation and are upregulated in response to anaerobic growth, such as the conditions seen in the biofilm environment (52). However, a number of studies have identified *ica*-independent biofilm formation and have questioned the in vivo importance of the *ica* locus (53-55). This locus is regulated with the reversible inactivation by insertion sequence (IS256) phase variation in *S. aureus* and *Staphylococcus*

epidermidis (56,57). Other levels of control in *S. epidermidis* are accomplished through IcaR-mediated transcriptional repression (relieved by ethanol stress) and the *sigB* operon product σ^B (regulated by operon genes *rsbU* and *rsbV*) (57,58). Jefferson et al. (59) recently discovered that IcaR binds to a 42-bp region just upstream of *icaA*, and they hypothesize that its role is to sterically hinder the binding of σ^B , thus preventing activation of the *ica* locus. Since a number of insertion sequences (e.g. IS1181 and IS431), *icaR* homologues, and the *sigB* operon have been found in most *S. aureus* strains (e.g., Mu50, N315, MW2, and Newman), these regulatory mechanisms may also hold true.

In *S. aureus*, several virulence factors are σ^B -regulated genes, including clumping factor, fibronectin-binding protein A, and coagulase (60,61), all of which are positively controlled, as well as alpha- and beta-hemolysin, enterotoxin B, SplA (a serine protease), cysteine protease (SplB), the metalloprotease Aur, staphopain, and leukotoxin D, all of which are negatively regulated (62). Thus, the genes needed for attachment and biofilm formation are upregulated by σ^B . Also, it has been shown that IcaR is a strong negative regulator of the *ica* locus, as deletion of *icaR* augmented PIA production by nearly 10-fold and increased transcription of the *ica* locus approximately 100-fold (63). Another gene, *rbf*, has recently been identified by transposon mutagenesis. The Rbf protein was shown to be important in multicellular aggregation during biofilm formation, and also in the induction of biofilm formation by NaCl and glucose, but had no effect on *ica* transcription (64).

In addition to PIA, a number of other studies have elucidated vital genes and their products in the development of staphylococcal biofilms. There is recent evidence that attachment of bacterial cells to a polymer surface, a prerequisite for biofilm formation, may be promoted by an autolysin of *S. epidermidis* (65); the homologue in *S. aureus* (*atl*) may also function in this manner. A two-component regulatory gene locus that mediates adhesion and influences biofilm formation in *S. aureus* has also been studied recently. This locus is a system encoded by *arIRS*, a member of

the OmpR-PhoB family of response regulators that is regulated by the *agr* and *sarA* loci (66,67). When upregulated, the product of *arlS* prevents biofilm formation and may mediate attachment to polymer surfaces by affecting peptidoglycan hydrolase activity. Teichoic acid structure is also crucial in the development of biofilms. Specifically, the addition of D-alanine esters to teichoic acids via *dltA* may be an important factor in imparting the proper charge balance on the gram-positive cell surface, enabling initial attachment and subsequent biofilm formation. Another *S. aureus* gene, for biofilm-associated protein (Bap), which was required for biofilm formation on inert surfaces, was discovered via transposon mutagenesis. However, because only 5% of bovine mastitis isolates and none of the 75 clinical isolates tested possessed the coding sequence for Bap, the in vivo significance of this protein may be doubtful. In another study, the differential gene expression in planktonic (shaken) versus biofilm (static) *S. aureus* cultures was evaluated, and five genes whose expression levels were increased in biofilms were identified (68). These were the gene encoding threonyl-tRNA synthetase (upregulated by amino acid starvation), three oxygen starvation response genes, and a stress response gene that encodes the ATPase ClpC. Another study employing microarrays to study differential gene expression between these conditions found 48 genes that were enhanced at least two-fold in the biofilm compared to planktonic conditions (69). As mentioned above, research has shown that biofilm formation is upregulated by anaerobic, osmotic, and ethanol stresses due to the stress-induced alternative sigma factor, σ^B (70).

Conclusion

Osteomyelitis continues to be a serious issue in the United States and throughout the world. These infections commonly occur in the long bones of the legs, but may also be found in the spine, the feet, and even in the base of the skull. Contiguous-focus osteomyelitis is commonly seen in patients with diabetes mellitus and often involves polymicrobial infections in the feet, while hematogenous osteomyelitis often occurs in the long bones, commonly in

children. Risk factors, such as systemic or local immunocompromise and prosthetic implantation, can increase one's risk for infection. *S. aureus* is the most commonly isolated organism in osteomyelitis cases. Biofilm formation by this pathogen and other microbial species responsible for osteomyelitis allow immune evasion, as well as resistance to clearance by antimicrobial agents. The only way to successfully treat such infections is to surgically remove the diseased tissue. This pathogen is re-emerging as its resistance to almost every available antibiotic is quickly becoming a reality. Once confined to the hospital setting, MRSA is now also causing life-threatening infections in the community. This is particularly troubling, as the individuals becoming infected in the community often are fully immunocompetent. Therefore, there is a need for a better understanding of the properties of *S. aureus*-mediated diseases, including the biofilm mode of growth, and the development of novel therapeutics to combat these infections.

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