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REVIEW

Staphylococcal Osteomyelitis: Disease Progression, Treatment Challenges, and Future Directions

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SUMMARY Osteomyelitis is an inflammatory bone disease that is caused by an infecting microorganism and leads to progressive bone destruction and loss. The most

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common causative species are the usually commensal staphylococci, with *Staphylococcus aureus* and *Staphylococcus epidermidis* responsible for the majority of cases. Staphylococcal infections are becoming an increasing global concern, partially due to the resistance mechanisms developed by staphylococci to evade the host immune system and antibiotic treatment. In addition to the ability of staphylococci to withstand treatment, surgical intervention in an effort to remove necrotic and infected bone further exacerbates patient impairment. Despite the advances in current health care, osteomyelitis is now a major clinical challenge, with recurrent and persistent infections occurring in approximately 40% of patients. This review aims to provide information about staphylococcus-induced bone infection, covering the clinical presentation and diagnosis of osteomyelitis, pathophysiology and complications of osteomyelitis, and future avenues that are being explored to treat osteomyelitis.

KEYWORDS *Staphylococcus aureus*, *Staphylococcus epidermidis*, antibiotic, joint infections, nonantibiotic, osteomyelitis

INTRODUCTION

Osteomyelitis, translated from Greek, means inflammation of the bone marrow (*osteon*, bone; *myelos*, marrow; and *itis*, inflammation) (1). The disease can be restricted to a single portion of the bone or affect several regions, such as the marrow, cortex, periosteum, and/or surrounding soft tissue (Fig. 1) (2). Osteomyelitis is often classified by the location within the bone, extent of dispersion, and source of infection. Although it can be caused by a variety of pathogens, it is most commonly caused by the opportunistic Gram-positive staphylococci (approximately 75% of cases, collectively) (3), which can originate from the blood (hematogenous source) or contiguously.

Microbiology of Staphylococci

Staphylococci are Gram-positive bacteria that have a round morphology and a diameter of 0.5 to 1.8 μm. The cell wall is what attributes the term "Gram positive" to staphylococci and is composed of layers of peptidoglycan, lipoteichoic acids, and teichoic acids (4). There are more than 20 different staphylococcal species described in *Bergey's Manual of Systematic Bacteriology* (5); however, *Staphylococcus aureus* and *S. epidermidis* are the most significant in regard to human interactions (6). *S. aureus* and *S. epidermidis* are usually commensal inhabitants of the skin microflora and mucosal surfaces. Approximately 20% of healthy individuals are permanently colonized asymptomatically by *S. aureus*, with 70% of individuals either transiently or not colonized (7). However, *S. aureus* has adapted to become a perilous human pathogen causing a variety of diseases, ranging from suppurative infections, such as boils, to more life-threatening infections, such as septicemia (8). In addition to a thick cell wall, around 80 to 90% of *S. aureus* strains possess a capsule which provides protection for the bacterium, as it has antiphagocytic properties due to the host's inability to recognize the invading microorganisms (9). Notably, *S. aureus* strains that are capsule negative have been shown to induce chronic infection in mouse models due to their ability to survive intracellularly (10). The ability of *S. aureus* and *S. epidermidis* to colonize and cause host infection is attributed primarily to the presence of various cell wall-anchored (CWA) proteins and extracellular factors. Several of these proteins can adhere to host cells and/or extracellular matrix (ECM) molecules and have been termed microbial surface components recognizing adhesive matrix molecules (MSCRAMM) (9). Examples of MSCRAMMs include fibronectin binding proteins (FnBP) and collagen adhesin (Cna) (11). Once colonized, staphylococci can secrete toxins which aid in invasion and dissemination throughout the host. Nearly all strains of *S. aureus* and *S. epidermidis* secrete the four hemolysins (alpha, beta, gamma, and delta), lipases, proteases, hyaluronidase, nucleases, and collagenase. The main functions of these toxins are to break down the host tissue and provide nutrients for bacterial survival and growth (12, 13).

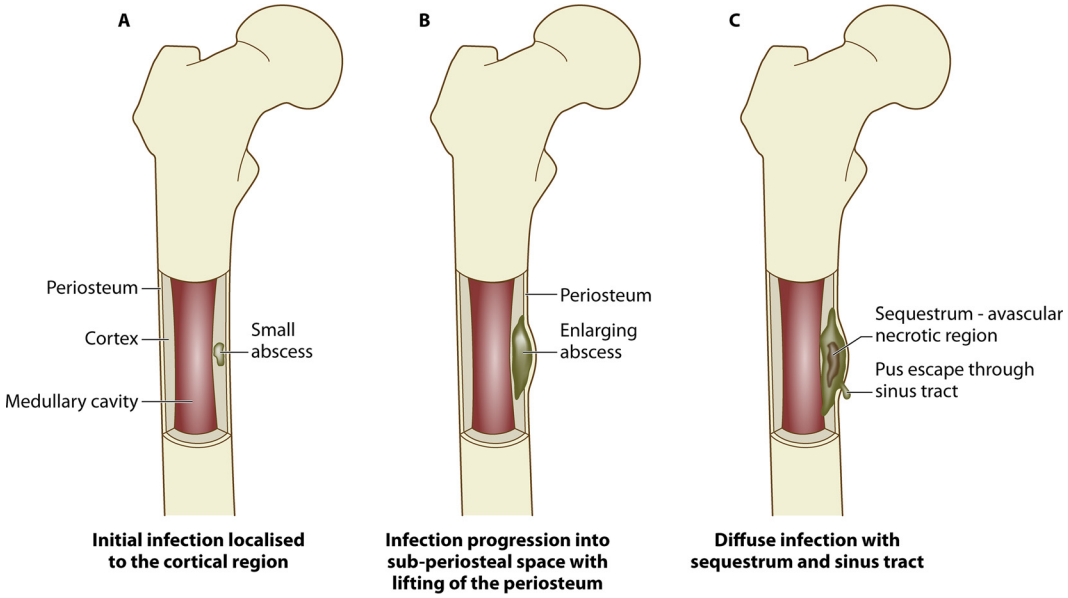

FIG 1 Progression of osteomyelitis. An abscess develops from a localized infection that constricts the blood flow to the area (A), resulting in an avascular region of necrotic bone tissue called the sequestrum (B), followed by development of new bone surrounding the sequestrum, termed the involucrum, which may also have a sinus tract through which purulence can escape (C).

MODES OF BONE INFECTION

There are many contributing factors that predispose a patient to developing osteomyelitis, including age, diabetes, peripheral vascular disease, intravenous (i.v.) drug use, surgical implants, and immunodeficiency due to disease or immunosuppressant drugs (14). The causative organisms in osteomyelitis can originate from either hematogenous or contiguously spread sources, often referred to as endogenous or exogenous sources, respectively (15).

Hematogenous Osteomyelitis

Hematogenous osteomyelitis is usually monomicrobial (16). It occurs most commonly in patients lacking any prior risk factors or infection; however, it can also be caused by the seeding of circulating pathogens in the blood, which can arise from an existing infection. Hematogenous osteomyelitis represents just 20% of all osteomyelitis infections; however, the majority of osteomyelitis cases in children are hematogenous (85% of cases for patients under 17 years of age) (15).

Contiguous Spread of Infection

In contrast to hematogenous osteomyelitis, contiguous spread of infection is most often polymicrobial and most commonly affects adults (17–19). Contiguously spread osteomyelitis can originate from trauma, direct inoculation during operative procedures, or surrounding infected soft tissues.

It is estimated that half of osteomyelitis cases in adults are due to trauma (20). Trauma can result in either open or closed fractures (presence or absence of exposed bone). Damaged connective tissues, including skin, muscle, and bone, expose proteins to which bacteria readily bind, such as collagen and fibronectin, increasing the chance of inoculation (21). In a clinical study carried out by Merritt, up to 1 in 5 patients who acquired open fractures were reported to have developed infections (22).

People with soft tissue infections who develop underlying infection of the bone are most commonly over the age of 40 and have diabetes mellitus (23). Osteomyelitis spreading from diabetic ulcers due to neuropathy and vascular insufficiency most commonly occurs in the bones of the feet: the toes, metatarsal heads, and calcaneum (24). According to Malhotra et al. and Lavery et al., 12 to 20% of those with diabetic foot

ulcers develop an infection of the underlying bone (25, 26), and in severe cases of foot ulcers this prevalence can be higher than 66% (27).

DEVELOPMENT OF ACUTE AND CHRONIC BONE INFECTIONS

The pathology of osteomyelitis is characterized by severe inflammation, impairment of vasculature, and localized bone loss and destruction. In an attempt to overcome the infective microorganisms, leukocytes produce inflammatory cytokines and enzymes that break down the infected and surrounding tissue (28). Purulence consisting of dead leukocytes and host/bacterial cells can fill intercellular spaces around the infection and form an abscess. In chronic infection, abscesses can impair blood flow and strip the periosteum, creating an area of vascularized, necrotic bone called a sequestrum (29). Vascular impairment makes the foci of chronic infection impervious to the immune system and systemic antibiotics. The sequestrum is indicative of a chronic infection and compromises the bone's integrity. Often the formation of new bone—an involucrum—occurs, which forms from remaining intact fragments of the periosteum and functions to provide axial support to weight-bearing bones and prevent pathological fracture (14, 30). Exudate or purulence from the infection may escape through an opening in the bone called a sinus tract (Fig. 1).

CLINICAL PRESENTATION AND DIAGNOSIS

Diagnosing osteomyelitis is often a difficult challenge, as there are vast variations in clinical presentation. Early diagnosis is the key to the successful treatment of osteomyelitis. Schmidt et al. developed a diagnostic tool for osteomyelitis that uses a scoring system based on clinical, laboratory, and technical information (31). The scoring system is based on (i) clinical history and risk factors; (ii) clinical examination and laboratory test results, including leukocyte counts and detection of inflammatory markers, such as via the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) level; (iii) diagnostic imaging, such as ultrasound, radiology, computed tomography (CT), or magnetic resonance imaging (MRI); (iv) microbiology analysis; and (v) histopathology. Unfortunately, many of these individual diagnostic methods lack specificity and sensitivity and are associated with many issues, as Tiemann et al. outlined (32). Lab test results involving leukocyte counts and inflammatory markers are often not reliable. For example, in a review by Scott et al., 41% of patients who presented with acute hematogenous osteomyelitis presented with a leukocyte count of $<$10,500, which is within the normal range of $\sim$4,500 to 11,000 (33). In up to 40% of osteomyelitis cases, microbiological tests produce false-negative results. This may be due to the difficulty in culturing the causative organism secondary to location, inability of the patient to undergo surgical intervention, or the fact that the patient may have been started on antibiotics prior to the collection of a specimen for culture, thus altering the results of laboratory testing. In addition, diagnosing osteomyelitis through imaging methods is often delayed because bone necrosis is difficult to detect by plain radiography until up to week 3 of infection, with a reported positive diagnosis rate of only 20% after 2 weeks (21).

OSTEOMYELITIS CLASSIFICATION

As osteomyelitis is a heterogeneous disease, the large variation in patient populations along with a number of factors critical for guiding an appropriate treatment strategy has resulted in more than 12 different classifications. While none of the classifications are ubiquitously accepted, two classifications are widely used because they provide information on the nature and origin of the disease while taking into account the patient's physiological status, parameters deemed critical in osteomyelitis. Any type of osteomyelitis can develop from the acute stage and continue into the chronic stage of the disease (34). Prescription of treatment for osteomyelitis in the clinical setting largely depends on the classification as either "acute" or "chronic." Although there is often much difficulty in this classification, the degree of tissue injury is generally directly correlated with the disease stage (35). Throughout the literature,

TABLE 1 Major classification systems used for diagnosis of osteomyelitis[a]

Cierny-Mader Classification	
Anatomic Type	
Type I	Medullary osteomyelitis
Type II	Superficial osteomyelitis
Type III	Localized osteomyelitis
Type IV	Diffuse osteomyelitis
Physiologic Class	
A-Host	Good immune system and delivery
B-Host	Compromised locally (B^L) or Systemically (B^S)
C-Host	Requires suppressive or no treatment; minimal disability; treatment worse than disease; not a surgical candidate
Clinical Stage	
Type + Class = Clinical stage	Example: Stage IVB^S osteomyelitis – a diffuse lesion in a systemically compromised host

Waldvogel Classification	
Osteomyelitis Stage	
Acute	Initial admission for the same disease
Chronic	With history of admission for the same disease
Source of Infection	
Haematogenous osteomyelitis	
Osteomyelitis associated with peripheral vascular disease	
Osteomyelitis secondary to a contiguous focus of infection	

[a]The Waldvogel system was adapted from reference 16; the Cierny-Mader classification was reproduced from reference 36 with permission of Springer Science and Business Media.

there are a number of detailed guidelines published to classify the infection, the most highly cited of which are the Waldvogel system and the Cierny-Mader system (16, 36).

Waldvogel Classification

The Waldvogel classification system (Table 1) defines the infection as either acute or chronic based on the persistence of infection, and the infection is subsequently classified based on the source of infection (16). Waldvogel et al. found that this definition not only showed evidence of differences in clinical presentation but also improved the disease cure rate.

Cierny-Mader Classification

The Cierny-Mader classification system (Table 1) is based on four key factors: the condition of the host, the functional impairment caused by the disease, the site of involvement, and the extent of bony necrosis. It does not deem it necessary to distinguish between acute and chronic infections. In this classification system, the anatomic type of osteomyelitis (I to IV) is added to the physiologic class of the patient (A, B, or C), which results in one of the 12 clinical staging systems of adult osteomyelitis ($I^{A,B,C}$, $II^{A,B,C}$, $III^{A,B,C}$, and $IV^{A,B,C}$). From this staging system, the osteomyelitis treatment is derived, including debridement strategies, dead space management, and antibiotic administration. Cierny et al. state that using these four key factors allows comparison of new treatment protocols and the effectiveness of new therapeutic modalities (36).

PATHOPHYSIOLOGY OF OSTEOMYELITIS

Bone as a Target Organ

Bone is a dynamic connective tissue that is constantly being remodeled and

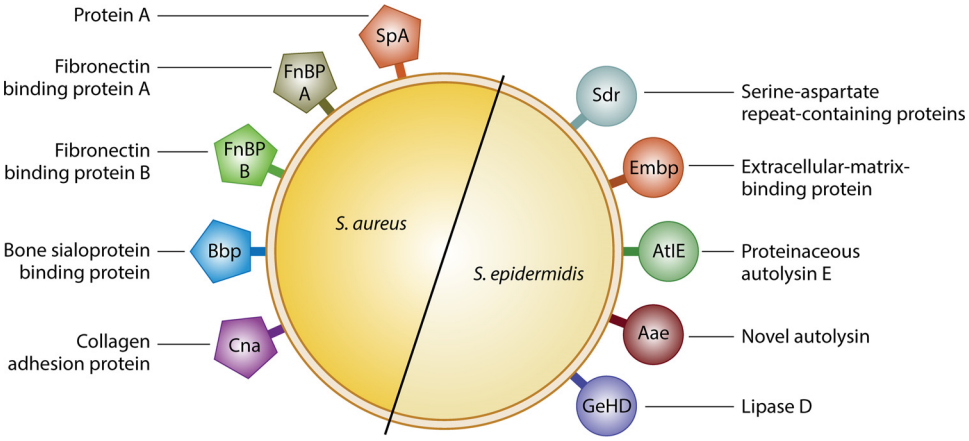

FIG 2 *Staphylococcus aureus* and *Staphylococcus epidermidis* cell surface proteins, known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), that are involved in interacting with bone and the bone ECM.

renewed under the governance of three main bone cells: osteoblasts, osteocytes, and osteoclasts. Osteoblasts are the bone-forming cells, derived from mesenchymal stem cells (MSC) in the bone marrow, and are responsible for producing the main organic extracellular matrix (ECM) components of bone. When osteoblasts are fully mature cells, they produce osteoid—unmineralized organic bone matrix—in the form of a membrane-bound vesicle (37). Osteoid consists of collagenous and noncollagenous proteins. Collagen type I makes up 90% of the osteoid, with the remainder comprised of proteins, such as proteoglycans (38) and glycoproteins. Common glycoproteins found in the ECM include fibronectin, osteonectin, osteopontin, bone sialoprotein, and osteocalcin (39, 40). When osteoblasts generate and fully immerse themselves in ECM, they become osteocytes—terminally differentiated osteoblasts. Osteocytes have been implicated in directing the bone remodeling process through their ability to respond to bone loading and detection of microcracks. Osteoclasts are the bone-resorbing cells, which operate by decalcifying hydroxyapatite and degrading organic ECM. Osteoclasts work in harmony with osteoblasts to retain bone remodeling homeostasis. Notably, an imbalance in the activity between these cells can result in altered bone morphology and pathological bone (41–43). When bone is exposed to the external environment, bone cells and the ECM are ideal colonizing targets of microbes, in particular staphylococci, which have the MSCRAMMs and anchoring proteins to colonize bone (44).

Staphylococcal Colonization of Bone

Once staphylococci have accessed the bone, the first step to colonization is primary attachment. Attachment is facilitated by the presence of MSCRAMMs and other cell wall-anchored proteins on staphylococci (Fig. 2). Colonization of bone can occur through direct interaction with the bone cells, plasma proteins, or the ECM. Once colonized, staphylococci can produce a biofilm, which facilitates persistence of the infection (45, 46). Biofilms are organized communities of microorganisms enveloped in an extracellular matrix attached to a surface (47–49). Additionally, staphylococci can also produce toxins, many of which facilitate dissemination throughout the host, allowing recolonization and reinfection (50).

***Staphylococcus aureus*.** In *S. aureus*, there are multiple MSCRAMMS and CWA proteins important for the pathogenicity of infection, including protein A (SpA), fibronectin binding proteins A and B (FnBP A/B), bone sialoprotein binding protein (Bbp), and collagen adhesion protein (Cna) (Table 2). In addition to being anchored to *S. aureus*'s cell wall, SpA can also be secreted. Although the primary function of SpA is immune evasion, studies have documented its direct role in bone infection. It was shown that SpA can directly bind to osteoblasts, mediating cell death, inhibition of

TABLE 2 Protein interactions involved in progression and pathogenicity of staphylococcal infection

Organism	MSCRAMM(s)	Matrix/bridge protein(s)	Receptor[a]	Functional response	Reference(s)
S. aureus	Collagen adhesion (CNA)	Collagen	NA	Colonization	68, 201
	Bone sialoprotein (Bbp)	Fibrinogen	Bone sialoprotein	Unknown	67, 202
	Fibronectin binding proteins (FnBP)	Fibronectin	$\alpha5\beta1$	Internalization	58–60
	Staphylococcal protein A (SpA)		TNFR1	Induction of bone loss (apoptosis) and bone destruction (osteoclastogenesis), inhibits mineralization	51–54
S. epidermidis	Serine-aspartate repeat-containing proteins (Sdr)	Fibrinogen, collagen	NA	Colonization	13, 78, 79, 82
	Extracellular matrix binding protein (Embp)	Fibronectin	NA	Colonization	83, 203, 204
	Autolysin E (AtlE)	Vitronectin	NA	Colonization	84
	Autolysin adhesion protein (Aae)	Vitronectin	NA	Colonization	85
	GehD lipase	Collagen	NA	Colonization	86, 205

[a]NA, not available.

bone formation (osteogenesis), and induction of bone resorption (osteoclastogenesis) (51–54). The importance of osteoclastic activity in osteomyelitis is becoming evident, and therefore many studies have emerged to examine the effects of *S. aureus* in promoting osteoclastogenesis and osteoclastic activity. Osteoblast inhibition and osteoclast activation were also described by Kim et al., who demonstrated an induction of proinflammatory cytokines by activation of Toll-like receptor 2 (TLR2) in osteoblasts, resulting in production of RANKL. This in turn activated osteoclast differentiation, facilitating bone resorption in mice lacking TLR2 and demonstrating the hallmark presentations seen in osteomyelitis (44). Activation of osteoclasts through various cellular pathways was also recently documented, with protein A once again being a key player in this process (54, 55). If *S. aureus* does not interact directly with the cell, its FnBPs facilitate binding to host plasma proteins, such as fibronectin and fibrinogen, which can act as bridging molecules between the bacterium and the host cell receptors (56, 57). Additionally, when these FnBPs, specifically FnBPA and FnBPB, interact with fibronectin, it can cause internalization via the $\alpha_5\beta_1$ receptor on osteoblasts (58–60). Activation of this integrin results in the recruitment of molecules, such as tensin and talin, which interact directly with the cellular cytoskeleton. These molecules in turn cause the recruitment of tyrosine kinases, which initiate phosphorylation of the cytoskeleton and thus uptake of the bacteria (61). Internalization can lead to two outcomes: apoptosis of the cell or persistence of infection intracellularly. Apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) occurs due to it binding to its receptor on the osteoblast membrane. There are 5 known receptors of TRAIL: death receptors DR5 and DR4, decoy receptors DcR1 and DcR2, and soluble receptor OPG. Binding of TRAIL to these receptors leads to the activation of caspases 8 and 10 (62). When activated, these then cleave caspase 3, which results in cellular apoptosis via mitochondrial dysregulation (63). Additionally, intracellular *S. aureus* can activate interleukin-6 (IL-6), IL-12, and colony-stimulating factor (CSF), further contributing to bone destruction (64, 65). If *S. aureus* persists intracellularly, it will not activate this pathway, as discussed in more detail in the sections on complications of osteomyelitis. Internalization is not unique to osteoblasts and is generally seen as a mechanism of immune evasion. However, studies have demonstrated that *S. aureus* can interact with cells and not cause cell death but become internalized by bone marrow-derived macrophages, causing differentiation into mature osteoclasts as well as activation of noninfected osteoclasts (66). *S. aureus* is also equipped to interact with the bone ECM through Cna and Bbp. Notably, Cna is the only identified *S. aureus* cell surface protein that binds to collagen, whereas Bbp has been documented to bind both bone sialoprotein (BSP) and fibrinogen (67, 68). Note that it has been shown that MSCRAMMs

TABLE 3 Toxins and exoproteins involved in progression and pathogenicity of staphylococcal infection

Organism	Toxin(s) or exoprotein(s)	Functional response	Reference(s)
S. aureus	Toxic shock syndrome toxin 1 (TSST-1)	Activates osteoclastogenesis	74
	Alpha-hemolysin (Hla)	Osteoblast/osteoclast cell death	75
	Panton-Valentine leukocidin (PVL)	Persistence of infection	76, 206
	Canonical coagulase (Coa)	Inhibits osteoblast function	77
	Phenol-soluble modulins (PSMs)	Osteoblast cytotoxicity	207–209
S. epidermidis	PSMs	Osteoblast cytotoxicity and biofilm dispersal	90, 210

give *S. aureus* the ability to invade various mammalian cells in addition to bone cells (58, 69–73).

Toxins play a major role in the progression and pathogenesis of osteomyelitis. *S. aureus* produces many toxins; however, toxic shock syndrome toxin 1 (TSST-1), hemolysins (Hla), Panton-Valentine leukocidin (PVL), coagulase, and phenol-soluble modulins (PSMs) are known to contribute to the severity of bone infection (Table 3). TSST-1 is known as a superantigen whose primary function is to inhibit the host immune response. It was recently shown to activate osteoclasts, increasing bone resorption through an unknown novel mechanism and contributing to the weakening of the bone (74). Interestingly, however, this superantigen was not shown to be cytotoxic to osteoclasts. Hla is one of the most studied cytotoxins produced by *S. aureus* due to its prevalence among different strains and its toxicity toward a wide range of mammalian cells. Hla lyses red blood cells by forming pores in the cell membrane, facilitating the spread and dissemination of infection through tissues. In persistent bone infection, *hla* is downregulated, therefore contributing to the quiescent and latent nature of recurrent osteomyelitis (75). PVL is produced by only a small percentage of *S. aureus* strains (approximately 2 to 3%) but is associated with persistence and rapid extension of osteomyelitis in murine models, leading to extensive spread of the infection (76). The primary role of coagulase is to convert fibrinogen to fibrin, thus providing a fibrin coating on the surface of *S. aureus*, protecting it from the host immune response. Coagulase also aggravates bone destruction and bone loss in mouse models of osteomyelitis by reducing osteoblast proliferation, inducing apoptosis, and decreasing mineralization (77).

***Staphylococcus epidermidis*.** *S. epidermidis* has not been studied as extensively as *S. aureus*; hence, only a limited number of MSCRAMMs have been identified in this species, to date. These are the serine-aspartate repeat-containing (Sdr) proteins, extracellular matrix-binding protein (Embp), proteinaceous autolysin E (AtlE), novel autolysin (Aae), and lipase D (GehD) (13) (Table 2). The most extensively studied cell wall protein in *S. epidermidis* is SdrG, which binds fibrinogen (78) and is known to bind to osteoblasts (79). SdrG is part of the Sdr family, which is also composed of SdrF and SdrH, which are expressed in most strains (80). SdrF has been shown to facilitate binding to collagen and is thought to be expressed in isolates from medical device infections (81). SdrG binds to fibrinogen (78, 82), Embp binds to fibronectin (83), AtlE and Aae bind to vitronectin (84, 85), and GehD and SdrF bind to collagen, facilitating the interactions between bone ECM/cells and bacteria (81, 86).

In addition to the cell surface-associated virulence factors, staphylococci also secrete exoproteins, which can be cytotoxic, to aid in infection and dissemination (Table 3). *S. epidermidis* is traditionally known to form biofilms rather than to secrete exotoxin, with toxin production mostly limited to PSMs. PSMs are short, amphipathic, detergent-like molecules that have a proinflammatory and sometimes cytolytic function (13, 87). Biofilms are surface-attached agglomerates of bacteria embedded in a sticky extracellular matrix that is highly resistant to the host immune response and antibiotics. *S. epidermidis* is well known to form biofilms on medical device implants, allowing for the persistence of infection. These device-related infections are commonly seen in orthopedic implants, with removal of the device often required to remove the infection (88,

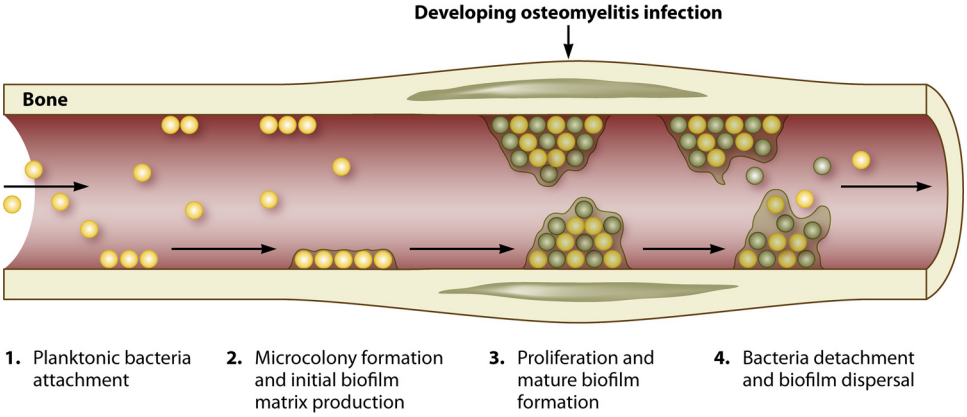

FIG 3 Stages of biofilm development (214). The first stage of biofilm formation in bone is attachment. Once attached, the bacteria begin to accumulate and produce a sticky matrix, which is the initial biofilm. This accumulation results in the formation of biofilm microcolonies and development of mature biofilm. The biofilm may then finally break down and release the bacteria from within, causing dissemination throughout the host.

89). Notably, the activation of biofilm production is conversely related to PSM production, suggesting that PSM-negative strains readily form biofilms (90).

COMPLICATIONS IN OSTEOMYELITIS TREATMENT

Persistent and Recurring Infections in Osteomyelitis

Staphylococcal biofilm development. The ability of bacteria to form biofilms is a natural mechanism. The stages of biofilm development are attachment, accumulation, and dispersal (Fig. 3). A number of factors mediate attachment, including Atl, teichoic acids, and MSCRAMMs (91), which allow positioning of the premature biofilm. The presence of human serum proteins alone enhances the expression of MSCRAMMs that promote biofilm formation (92). Once attached, the bacterial cells within the matrix multiply and accumulate, shaping the matrix surrounding them to include complexities such as water channels for nutrient and waste diffusion. It is thought that through quorum sensing governed by the *agr* system, bacteria are able to sense their environment and can disperse from the mature biofilm matrix and spread to other areas (49, 93). At present, there are two types of biofilm: (i) polysaccharide intracellular adhesion (PIA)/polymeric *N*-acetylglucosamine (PNAG)-mediated biofilm and (ii) a proteinaceous biofilm mediated predominantly by FnBPs and the major Atl protein (94, 95). In regard to *S. aureus*, methicillin-susceptible *S. aureus* (MSSA) isolates have previously been shown to produce PIA biofilm, with fewer invasive methicillin-resistant *S. aureus* (MRSA) isolates documented to produce the proteinaceous matrix due to the downregulation of the accessory gene regulator (Agr) system associated with expression of the methicillin resistance gene in MRSA isolates (95–97). Additionally, extracellular DNA (eDNA) released from both *S. aureus* and *S. epidermidis* is important for the adherence and accumulation of biofilms. Abolishment of AtlE, involved in eDNA release, resulted in a reduced capacity of the bacteria to form biofilms (18).

In chronic osteomyelitis, the ability of staphylococci to persist and reinfect is partially attributed to the development of biofilms. The presence of biofilms has been suggested as the main cause of clinical quiescence of chronic osteomyelitis. Biofilms can provide protection from the antibiotic arsenal, the host immune response, and shear stresses. Biofilms further enhance the survival of the staphylococci residing within them by functioning to seize and concentrate important environmental nutrients (18, 98).

As with most cases of chronic osteomyelitis, surgical intervention is usually required for removal of the sequestrum. The sequestrum has a decreased vascularity and oxygen tension, providing optimum conditions for bacterial attachment and biofilm formation. Debridement of the infected area would also include removal of the sequestra, as antibiotic therapy alone is unable to sufficiently penetrate the biofilm matrix and eradicate the infection within. Surgical revisions can result in infection relapse in up to

40% of cases; however, if the sequestrum remains present in the bone, it will facilitate spreading of the infection throughout the bone. Spreading of the infection will eventually result in the need for radical debridement and possible limb amputation (99, 100).

Persistent SCV staphylococci. In conjunction with the biofilm matrix, which provides protection for the bacteria within it, alterations of the bacterial metabolic activity which confer resistance to antibiotics are also observed. Persister cells and small-colony variants (SCVs) are found within biofilms and have been investigated extensively in the staphylococcal species (101, 102). SCVs have been described for osteomyelitis cases and have been deemed responsible for the recurrent infection associated with the disease due to their ability to survive intracellularly in a dormant state for many years, to then remerge as the parent strain and cause reinfection (103). Invasion and persistence of *S. aureus* in naturally nonphagocytic cells have been described for a range of cell types, including endothelial cells and keratinocytes (104, 105). One family of surface proteins found across the majority of *S. aureus* species are the FnBPs (e.g., FnBPA and FnBPB), which bind to the extracellular matrix protein fibronectin (106). It has been shown that these proteins not only can promote adhesion to surfaces but also can interact with naturally nonphagocytic cells and encourage uptake into the cell. Notably, Cna and Bbp favor FnBP internalization into nonprofessional phagocytic cells (44). This internalization has two possible outcomes: either the *S. aureus* invader activates production of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which in turn causes osteoblast apoptosis, or it can persist intracellularly as an SCV and cause recurrent infection months or years later (107, 108).

Antibiotic resistance. Antibiotic resistance is an international issue that affects both developed and developing countries. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) estimate that in both the European Union and the United States, more than 23,000 people die annually as a result of antimicrobial resistance, with *S. aureus* responsible for nearly 50% of those deaths. Antibiotic resistance can exacerbate staphylococcal infections by making them increasingly difficult to treat with antibiotics. There are three main mechanisms by which bacteria confer resistance: (i) changes in the membrane permeability/efflux of the antimicrobial, (ii) destruction of the antimicrobial compound, and (iii) alteration of the bacterial protein which is a target of the antimicrobial (109, 110). Efflux pumps present in bacteria can confer a natural resistance to antibiotics. These pumps are seen across both Gram-negative and Gram-positive bacteria, including *Escherichia coli* and *S. aureus*. When overexpressed, these pumps have the ability to transfer unwanted molecules from the cell (111, 112).

The direct inactivation of antibiotics via enzymatic strategies has been a major mechanism of antibiotic resistance since penicillin resistance emerged in the 1940s. Penicillinase, or β-lactamase, was shown to directly inactivate penicillin via hydrolysis of the β-lactam ring of the compound (113, 114). Since then, a multitude of enzymes have been identified that can degrade various classes of antibiotics, including β-lactams, aminoglycosides, phenicols, and macrolides (114).

Alteration of the bacterial target to prevent the interaction with the antibiotic is another mechanism by which resistance is conferred. There are two ways that this is possible: via a mutational change in the target protein or by a nonmutational modification of the target. An example of target change includes the acquisition of a gene homologous to the original target, such as that seen in *S. aureus* and *S. epidermidis*. In an effort to overcome the arsenal of β-lactams, *S. aureus* and *S. epidermidis* acquired a methicillin resistance gene, *mecA*, which is carried on a mobile heterogeneous genetic element called the staphylococcal cassette chromosome (SCC). The *mecA* gene encodes a penicillin binding protein, PBP2a, which displays decreased affinity for β-lactam antibiotics, allowing cell wall synthesis to occur as normal in the presence of the antibiotic. This can lead to the emergence of MRSA (115–118). MRSA is often isolated from bone infections and is usually treated with vancomycin, a glycopeptide that inhibits cells wall synthesis of *S. aureus* in a manner different from that for β-lactams.

However, vancomycin-resistant *S. aureus* (VRSA) was isolated in Japan in 1997, instilling concerns over the treatment of these infections globally (119).

Noninfectious Complications of Osteomyelitis

With the onset of infection, there are various complications related to the bone that are not directly related to the infection but are a result of the infection. As previously described, the presence of infection can result in the production of cytokines which activate the bone-resorbing osteoclasts. Additionally, the presence of infection causes osteoblast cell death, thus preventing new bone formation (51, 53). This weakens the bone, which can result in pathological bone fractures, further compounding the issue (120). Moreover, surgical debridement of the bone can also result in weakening of the bone, which may further result in bone fractures if the bone is not supported sufficiently or is loaded prematurely. In the case of vertebral osteomyelitis, neurological compromise has been described. This includes documentation of motor weakness, paraparesis, and even paralysis, all caused by abscess formation compressing various parts of the spine, such as the spinal cord and nerve root (121). In children, osteomyelitis at the growth plates of long bones may interrupt normal growth. Patients with a chronic draining osteomyelitic sinus are also at increased risk of development of a squamous cell carcinoma (122).

CURRENT TREATMENT STRATEGIES

Osteomyelitis therapy requires an interdisciplinary approach involving a combination of patient evaluation, antibiotic therapy, and surgical intervention (123–125). Once the diagnosis of staphylococcal osteomyelitis is established, there are several factors that need to be considered for effective treatment. Successful treatment will almost certainly depend on debridement of infected tissue and the surgical resection of any necrotic bone or prosthetic material. Fundamentally, necrotic bone is the hallmark of chronic osteomyelitis, and its presence necessitates surgical debridement prior to any successful antimicrobial treatment. The management of prosthetic joint infection is beyond the scope of this review, but this is well covered elsewhere (126). Fracture fixation may also be required. When surgery is not possible, the patient may require long-term (usually oral) antimicrobial suppression of the infection.

Osteomyelitis Treatment Guidelines

The 2013 Cochrane review of chronic osteomyelitis examined all randomized and quasi-randomized trials of different antibiotic regimens given after surgical debridement of chronic osteomyelitis and found only eight small applicable trials, with a total of just 282 patients (127). Most trials were over 20 years old and do not reflect the emerging prevalence of antimicrobial-resistant pathogens, which are becoming more and more commonplace in modern health care settings. The authors concluded that the quality and reporting of these trials were often inadequate.

The level of evidence for treatment of acute osteomyelitis in adults is even worse. There is little objective evidence for the accepted precepts of treatment, and large, high-quality trials are lacking. There are various pieces of advice on the duration and route of treatment, and confusion exists regarding the superiority of intravenous/parenteral treatment over oral treatment. There are no Cochrane reviews for the treatment of acute osteomyelitis in adults. There are no UK or ECCMID guidelines for the treatment of acute osteomyelitis in adults, although the Bone Joint Infection Committee for the Italian Society of Infectious Tropical Diseases (SIMIT) guidelines are published in English and can provide useful guidance to clinicians (128). There are widely accepted and used Infectious Diseases Society of America (IDSA) treatment practice guidelines for the treatment of prosthetic joint infection and vertebral osteomyelitis, but dedicated treatment guidelines for acute osteomyelitis are still awaited. The treatment of acute osteomyelitis can be difficult and is largely based on expert opinion.

Although *S. aureus* and *S. epidermidis* remain the commonest etiological agents of native bone and joint infections, empirical treatment of osteomyelitis should be delayed (where possible) until samples for culture are obtained to allow for optimal antimicrobial selection (129). The gold standard for diagnosis is bone biopsy (130). Having found an organism to treat, the results of susceptibility testing can then inform the choice of the optimal agent, route, and duration of treatment.

Antibiotic Selection

The agent selected for treatment should be guided by the antimicrobial susceptibility testing results. The most important susceptibility distinction is the oxacillin/methicillin susceptibility result, which defines whether methicillin-susceptible or -resistant *S. aureus* or *S. epidermidis* (MSSA/MSSE or MRSA/MRSE) is involved. If the organism has not been cultured but is detected by 16S rRNA gene PCR or another molecular method, then the susceptibility testing results may not be available, and treatment has to be planned on the basis of the resistance patterns detected from the staphylococci cultured from the patient's other sites or local epidemiology. One day, genome sequencing may possibly be used to provide a genotypic prediction of the organism's susceptibility pattern (131), but this is expensive and not available outside research labs at present. An indication of the success of the selected treatment method may be given by reductions in the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) level. The main treatment choices for both methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* all achieve therapeutic levels of bone penetration (132) and are shown in Table 4 (133, 134).

Route and Duration of Treatment

Since the paper of Waldvogel et al. in the *New England Journal of Medicine* in 1970 (135), a treatment duration of at least 4 weeks has commonly been advocated. This is based on Waldvogel et al.'s comparison of patient outcomes between two groups treated with "intensive" (more than 4 weeks) and limited therapy regimens. This rationale has been reiterated in recent years based on similar case series. Zimmerli published a meta-analysis of vertebral osteomyelitis trials and found no significant difference in outcomes for 22 different treatment regimens (136). Seven trials in *S. aureus* osteomyelitis from 1987 to 1999 showed no difference in outcomes between parenteral and oral antibiotics, but he noted that emerging resistance trends may render these outcomes clinically meaningless. He concluded that "although control trials are lacking, a treatment duration of 6 weeks is generally recommended."

However, antimicrobial choice should also be determined by the reported penetration of the chosen agent into bone. Extant data are drawn from animal models comparing bone and serum levels of drugs, but there is a lack of standardized methodology and standard assays, and performances may differ from animal bone to human bone and between diseased and healthy tissues (130). Further, antibiotic levels may differ between healthy/experimental tissue and diseased human bone due to the differences in the pH and oxidative microenvironment of infection (136). The commonly used animal models were first developed by Norden et al. in the 1960s, and these have contributed to our understanding of bone revascularization and remodeling in response to infection and debridement, but some of the drugs used in humans are toxic to animals or have a poor correlation between animal and human efficacies, and vancomycin (which is a commonly used agent in human treatment) performs poorly in rabbit models (137). Lew and Waldvogel (2) reviewed the treatment of acute osteomyelitis, and while they concluded that antibiotics should be given for 4 to 6 weeks and "if possible by the intravenous route," they did caution against the complications and risks associated with long-term intravenous catheters and a prolonged hospital stay. They concluded that "parenteral therapy remains the approach of choice until more comparative studies are completed" (16). However, there is an emerging body of opinion and evidence to challenge the dogma of 6 weeks of parenteral treatment. Spellberg and Lipsky questioned Waldvogel et al.'s case series and described it as

TABLE 4 Therapeutic options for treatment of *S. aureus* and *S. epidermidis* osteomyelitis[a]

Agent (class)	Dose	Methicillin susceptibility status	Interactions	Side effects	Comments
Recommended i.v. agents for treatment of *S. aureus* and *S. epidermidis* osteomyelitis					
Flucloxacillin (penicillin)	2 g q6h	MSSA/MSSE	No significant interactions	Rash, nausea, vomiting, diarrhea, cholestatic hepatitis	First-line treatment for MSSA/MSSE infection
Nafcillin (penicillin)	2 g q4h	MSSA/MSSE	Tetracyclines, warfarin	Phlebitis, rash, neutropenia, interstitial nephritis	First-line treatment for MSSA/MSSE infection
Oxacillin (penicillin)	2 g q4h	MSSA/MSSE	Tetracyclines	Phlebitis, rash, hepatitis	First-line treatment for MSSA/MSSE infection
Cefazolin (cephalosporin)	2 g q8h	MSSA/MSSE	Probenecid (increase in cephalosporin serum concn), warfarin	Phlebitis, rash, fever, eosinophilia	Convenient for OPAT
Ceftriaxone (cephalosporin)	2 g q24h	MSSA/MSSE	Calcium-containing solutions, probenecid (as described above), warfarin, lansoprazole	Pseudocholelithiasis, phlebitis, rash, fever	Convenient for OPAT
Vancomycin (glycopeptide)	15 mg/kg of body wt q12h	MRSA/MRSE	Nondepolarizing muscle relaxants, nephrotoxic agents	Nephrotoxicity, ototoxicity, thrombocytopenia, red man syndrome	Target trough of 15–20 mg/liter, consider combination therapy, may be less effective against strains with MICs of 1–2 μg/ml
Teicoplanin (glycopeptide)	12 mg/kg q24h	MRSA/MRSE	Nephrotoxic agents, ototoxic agents	Thrombophlebitis, rash, neutropenia, eosinophilia, ototoxicity	Target trough of $>$20 μg/ml
Daptomycin (cyclic lipopeptide)	6 mg/kg q24h	MRSA/MRSE	Statins	CK elevation, eosinophilic pneumonia	Monitor CK, convenient for OPAT
Oral treatment options for either MSSA/MSSE or MRSA/MRSE osteomyelitis (if isolates are susceptible)					
Levofloxacin (fluoroquinolone)	750 mg q24h	MSSA/MSSE, MRSA/MRSE	QTc-prolonging agents, warfarin	Diarrhea, phototoxicity, QTc prolongation, tendon rupture, seizures	Use combination therapy
Trimethoprim-sulfamethoxazole (antifolate)	DS 2 tabs q12h	MSSA/MSSE, MRSA/MRSE	ACE inhibitors, azathioprine, cyclosporine, folinic acid, *para*-aminobenzoic acid, phenytoin, sulfonylureas, oral contraceptives, warfarin	Nausea, vomiting, rash, hyperkalemia, bone marrow suppression	Consider combination therapy
Doxycycline (tetracycline)	100 mg q12h	MSSA/MSSE, MRSA/MRSE	Acitretin, barbiturates, bismuth salts, carbamazepine, digoxin, oral contraceptives, penicillins, warfarin	GI intolerance, photosensitivity, dental deposition	
Minocycline (tetracycline)	100 mg q12h	MSSA/MSSE, MRSA/MRSE	Acitretin, barbiturates, bismuth salts, carbamazepine, digoxin, oral contraceptives, penicillins, warfarin	Vertigo, ataxia, hypersensitivity pneumonitis, rash, GI intolerance, photosensitivity, dental deposition	Consider combination therapy
Linezolid (oxazolidinone)	600 mg q12h	MSSA/MSSE, MRSA/MRSE	SSRIs, MAOIs, tricyclic antidepressants, adrenergic agents, rifampin	Thrombocytopenia, anemia, optic neuropathy, peripheral neuropathy	Reserve for use when alternatives not available, monitor FBC
Clindamycin (lincosamide)	600 mg q6h (i.v.), 450 mg q6h (p.o.)	MSSA/MSSE, MRSA/MRSE	Erythromycin, kaolin-pectin, loperamide, nondepolarizing muscle relaxants	Diarrhea, nausea, vomiting, anorexia, rash	Check for inducible clindamycin resistance if erythromycin resistant
Rifampin (rifamycin)	300–450 mg q12h or 600 mg q24h	MSSA/MSSE, MRSA/MRSE	Numerous—check interactions when prescribing	Orange discoloration of urine, tears, and sweat, hepatitis, GI intolerance, flu-like syndrome	Use in combination therapy only, as *S. aureus* resistance develops quickly in response to monotherapy; particularly effective in treatment of biofilms and infected prosthetic material
Fusidic acid (fusidane)	500 mg q6h	MSSA/MSSE, MRSA/MRSE	Statins, ritonavir	Phlebitis, nausea, vomiting, diarrhea, elevated bilirubin	Use in combination therapy only, as *S. aureus* resistance develops quickly in response to monotherapy

(Continued on following page)

TABLE 4 (Continued)

Agent (class)	Dose	Methicillin susceptibility status	Interactions	Side effects	Comments
Newer i.v. agents with unproven but potential future role in treatment of MRSA osteomyelitis					
Ceftaroline (cephalosporin)	600 mg q8h	MRSA/MRSE	No significant interactions	Nausea, vomiting, diarrhea, crystalluria, elevated transaminases	Limited data, new agent with activity against MRSA/MRSE
Tigecycline (glycylcycline)	100-mg load, then 50 mg q12h	MRSA/MRSE	Oral contraceptives	Nausea, vomiting, hepatic failure, pancreatitis	Limited data, new agent with activity against MRSA/MRSE, spectrum may be excessively broad
Telavancin (lipoglycopeptide)	10 mg/kg q24h	MRSA/MRSE	QTc-prolonging agents, nephrotoxic agents	Nephrotoxicity, QTc prolongation, taste disturbances, nausea, vomiting	Limited data, new agent with activity against MRSA/MRSE
Dalbavancin	1,000–1,500-mg first dose, then 500 mg once a week	MRSA/MRSE	Unknown	Diarrhea, headache, nausea, abdominal pain, blood disorders, *Clostridium difficile* colitis, constipation, cough, fungal infection, oral candidiasis, phlebitis, pruritus, rash, urticaria, vomiting, vulvovaginal mycotic infection, red man syndrome	Limited data, new agent with activity against MRSA/MRSE

[a]Data are from references 133, 134, 137, and 211 to 213. Abbreviations: ACE, angiotensin-converting enzyme; CK, creatine kinase; FBC, full blood count; GI, gastrointestinal; i.v., intravenous; MAOI, monoamine oxidase inhibitor; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MSSE, methicillin-susceptible *Staphylococcus epidermidis*; OPAT, outpatient parenteral antimicrobial therapy; p.o., *per os*; SSRI, selective serotonin reuptake inhibitor; q6h, every 6 h.

retrospective, uncontrolled, heterogeneous, and based only on using penicillins as the treating agent (132). They stated that many oral agents now available can penetrate bone well and achieve levels in excess of the MICs, including agents with some action against susceptible strains of MRSA. They concluded that oral therapy is acceptable and simple, that any preference for parenteral treatment may be based "more on custom than evidence," and that no strong evidence supports 4 to 6 weeks of treatment. Daver et al. retrospectively reviewed a cohort of adults with *S. aureus* osteomyelitis and compared those who received more than 4 weeks of intravenous treatment (median treatment duration of 60 days) to a group receiving less than 4 weeks of treatment (median intravenous treatment of 12 days followed by 42 days of oral treatment) (138). The overall cure rate was 74%, with no significant difference between the groups. Several other studies have shown equivalent results between intravenous treatment and highly bioavailable oral treatment (127, 139, 140).

Clinicians are eagerly awaiting full publication of the OVIVA trial (oral versus i.v. antibiotics for bone and joint infection) (139). This large multicenter trial (>1,000 patients from >20 UK centers) is a randomized, noninferiority trial comparing oral and i.v. antibiotics for the duration of the patient's osteomyelitis treatment. Preliminary results presented at ECCMID 2017 demonstrated equipoise, reflecting the strongest evidence, to date, that carefully selected, highly bioavailable agents with good bone penetration are an appropriate therapy for bone and joint infections, relieving physicians of the long-held dogmas that intravenous therapy is paramount in the treatment of these infections. As well as facilitating early discharge from hospital, the oral route obviously avoids the potential complications of long-term indwelling venous access catheters.

Dead Space Management

After debridement of the infected site, there is an area left that is termed dead space. Dead space management typically involves harvesting autologous or autogenous bone grafts, most often from the pelvic iliac crest, followed by implantation into the defect site. Autologous bone grafts remain the gold standard for promoting healing, with almost 2.2 million procedures estimated per annum (133, 141). Grafts of this kind have optimal biological performance in terms of osteogenicity, osteoinductivity, and osteoconductivity (142). However, the use of autologous bone grafts is limited by considerable donor site morbidity, postoperative pain, and risk of infection and the lack of available tissue. Allogeneic bone grafts can also be employed, most commonly by transplantation of sterilized cadaverous bone. However, this is also restricted due to viral transmission and immune rejection issues (15, 143). Another method used to manage dead space is the use of muscle flaps. This method has several advantages, such as malleability, a dense capillary network, and encouragement of rapid collagen deposition. One study by Anthony et al. demonstrated a 96% success rate in 34 patients by use of this strategy (144). Drawbacks, however, include recurrent infection in cases of chronic osteomyelitis, which can result in infection of the muscle flap (145).

Local Antibiotic Delivery Strategies

The systemic administration of a sufficiently high dose of antibiotics to reach the necrotic region and clear the infection often results in toxicity. Therefore, a number of products focused on the local delivery of antibiotics to the site of infection while simultaneously regenerating bone have emerged in recent years (146–151). There are a range of products currently on the market (Table 5), which are typically classified according to the degree of biodegradability of the carrier and which vary with regard to material type, antibiotic type, and delivery method. Each technique ultimately aims to reduce the dependence on systemic antibiotics, decrease hospitalization costs, and, importantly, prevent late relapse, which is common in chronic osteomyelitis.

Nonbiodegradable antibiotic delivery systems are based on the acrylic material polymethylmethacrylate (PMMA), in the form of either cement (Palacos) or beads

TABLE 5 Commercially available bone-regenerative biomaterials, including collagen-based sponges and bone cement/beads, loaded with antimicrobials and used for treatment of osteomyelitis

Product	Company	Description	Indications
Collagen-based sponges			
Collatamp G/EG	EUSA Pharma	Resorbable collagen implant impregnated with gentamicin	Prevent and treat surgical site infections through local antibiotic delivery
Genta-Coll	Resorba	Hemostyptic collagen sponge containing gentamicin	Hemostasis in wounds when there is high risk of infection (including in osteomyelitis)
Septocoll E	Biomet UK Ltd.	Resorbable equine collagen fleece containing 2 forms of gentamicin (gentamicin sulfate [rapid release] and gentamicin crobefate [protracted release])	Potentially contaminated/contaminated wounds; revision operations in septic surgery
Bone cement/beads			
Septopal	Biomet UK Ltd.	PMMA chains loaded with gentamicin sulfate	Local drug delivery after surgical debridement
Stimulan	Biocomposites	Calcium sulfate (can mix with gentamicin, vancomycin, and tobramycin)	Complements dead space and infection management strategies (e.g., infected nonunions, osteomyelitis, and periprosthetic joint infection)
Palacos	Heraeus Medical	Bone cement (available with gentamicin)	Orthopedic replacement procedures

(Septopal). These can be combined with a number of antibiotics and have been used extensively in surgery to locally deliver antibiotics for the treatment of various musculoskeletal infections. Notably, this treatment is limited due to toxicity and the requirement for a thermally stable antibiotic (152). Additionally, PMMA products require removal, giving rise to the risk of reinfection. This drawback can be overcome by the use of biodegradable antimicrobial products.

Biodegradable delivery systems using calcium sulfate beads and collagen sponges with antibiotics have been in use for the past decade. These biodegradable delivery systems allow for the local delivery of antibiotics to the site of infection while providing a scaffold for the repair and regeneration of bone. Such products include Stimulan beads, which can be combined with a number of antibiotics, Collatamp G/EG (EUSA Pharma), and Genta-Coll (Resorba).

Nonantibiotic Antimicrobial Therapies

Current treatment strategies are continuously being researched and optimized, with many therapies, such as the Collatamp G/EG and Stimulan products mentioned above, reaching clinical settings. However, there are various limitations to these treatments, in particular targeting the infection. Thus, research into new and emerging technologies, such as nonantibiotic compounds, is an area of growing interest. A wide range of nonantibiotic materials, such as metals, polymers, and peptides, demonstrate antimicrobial activity (153–155). To date, these materials have been delivered by a variety of methods, including topically to the skin in the form of creams or bandages, as a coating on the surfaces of medical devices, or combined with other natural scaffolding materials and delivered locally to the site of infection, often reducing or even negating the use of antibiotics. Many of these nonantibiotic antimicrobial therapies are either clinically available or on the regulatory path toward product approval.

Metals. A number of metals, e.g., silver (156–158), iron (159), mercury (160), tellurium (161, 162), copper (163, 164), zinc (21, 165, 166), and lead (167), have been shown to possess antimicrobial properties. In contrast to antibiotics, metals do not pose the risk of decomposition and can usually be processed at high temperatures (168). The mechanisms by which metals target microbes are only partially known; it is thought that some metals kill microbes by ion penetration, which inactivates microbial enzymes, while others impair membrane function or produce reactive oxygen species (167, 169). They have even been shown to be potential antimicrobial agents against drug-resistant bacteria, including MRSA and MRSE (170).

Polymers (chitosan). Chitosan is a positively charged linear polysaccharide that is found naturally, most commonly derived from the shells of crustaceans. It is biodegradable, biocompatible, and nontoxic and displays antimicrobial activity (171). The molecular weight and degree of deacetylation of chitosan are said to affect its antimicrobial activity (172, 173). Chitosan also has excellent metal binding properties, as it is a chelating agent, and it is often combined with metal ions, such as the ions discussed above, to increase its antimicrobial activity against bacteria, including *S. aureus* (including MRSA) and *S. epidermidis* (174, 175).

Peptides. Antimicrobial peptides (AMPs) are short proteins ($<$50 amino acids) that form part of the human innate immune system and are secreted by leukocytes, epithelial layers in the skin, and various mucosal membranes (176, 177). Some antimicrobial peptides, e.g., LL-37, demonstrate broad antimicrobial activity along with the promotion of bone regeneration (178, 179). LL-37 has also been shown to inhibit both the binding and biofilm-forming abilities of *S. epidermidis* (180) and has demonstrated effectiveness against both extracellular and intracellular *S. aureus* isolates (181). There are currently 2,707 peptides in the Antimicrobial Peptide Database reported to have antimicrobial activity derived from a variety of sources, including bacteria, archaea, protists, fungi, plants, and animals (182).

CONCLUSIONS AND FUTURE PERSPECTIVES

Staphylococcus-induced osteomyelitis is a major clinical challenge, as current treatment strategies are suboptimal for tackling both the infection and restoration of the affected bone. The pathogenesis of this disease is a double-edged sword whereby not only can staphylococci utilize bone for colonization, but bone itself can facilitate infection progression. Moreover, in addition to the ability of staphylococci to adapt to and evade the immune response by using the host's own machinery, they have also acquired resistance mechanisms to survive a plethora of antibiotic treatments available today. This, in conjunction with the need for surgical intervention, has led to new, exciting approaches in the field. For example, there has been a shift toward developing bifunctional bone-regenerative biomaterials whose degradation matches the native bone regeneration rate, combined with local delivery of antibiotics (183–185). Controlling the release of antimicrobials, which functions both to minimize systemic toxicity and to reduce the risk of inducing antibiotic resistance by ensuring that the release dose and rate are above minimum bactericidal concentrations sufficient for total infection clearance, has also become a hot topic in the drug delivery field. This may be achieved through methods such as microparticle incorporation or surface adsorption, with an on-demand release responsive to infection development (pH change, presence of bacterial toxins, or raised temperature) possible (186–189). Although nonantibiotic antimicrobials may be second to antibiotics at infection clearance, they do have the added advantage of overcoming some of the resistance mechanisms developed by bacteria (190–192). These nonantibiotic antimicrobial-loaded materials may be used for infection prophylaxis, perhaps after orthopedic procedures, which may be lengthy, post-implant removal, or following bone debridement if there is an infection risk.

Another exciting research avenue is the development of new methods to target infection by using a more tailored approach. One such area is the use of clustered regularly interspaced palindromic repeats (CRISPR). CRISPR technology has gained much attention for its gene editing abilities, mainly in mammalian cells (193, 194). However, there has been considerable research into the use of CRISPR for the treatment of infectious diseases (195). Seminal research by Bikard et al. demonstrated the potential to use CRISPR/Cas9 in targeting staphylococcal infection by targeting the methicillin resistance gene in *S. aureus*, making a MRSA isolate susceptible to methicillin once again (196). Moreover, when the technology was delivered *in vivo*, there was a moderate, albeit significant reduction in infection in mouse models of *S. aureus* infection. This research demonstrates the potential use of CRISPR/Cas9 *in vivo*, advancing the field toward a more targeted and selective approach to treat infections.

Currently, the majority of biological processes understood today are conducted in a two-dimensional (2D) setting. However, there is an increasing need for more physiologically relevant models (197). Studies using three-dimensional (3D) models over the past 2 decades have bridged the gap between 2D cell culture and *in vivo* culture (198, 199). The development of collagen-based scaffolds for tissue regeneration has presented a new focus for studying bone infection. Research from our group has demonstrated that staphylococcus-induced bone infection results in hypermineralization of the osteoblasts, correlating with increased metabolic activity, when the bacteria are cultured in a 3D bone matrix (N. Kavanagh, F. J. O'Brien, and S. W. Kerrigan, submitted for publication). This has not been demonstrated previously, therefore highlighting the importance of using more physiologically representative models to study infection. Using such 3D models will help us to elucidate and understand disease progression and thus inform our decisions for translating into *in vivo* models.

To conclude, staphylococcus-induced bone infection requires extensive research, with a particular focus on the molecular mechanism adopted by staphylococci to cause infection. Development of physiologically relevant models, such as the 3D model developed by our group, is an important part of driving knowledge forward within the field. As a result, incorporating new emerging technologies into the scaffold, such as CRISPR, to treat the infection provides an exciting new platform for not only regener-

ating the affected area but also treating the infection in a tailored and selective manner, avoiding the perils of antibiotic-based treatments currently seen in osteomyelitis patients.

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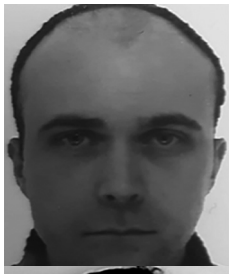

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Steven W. Kerrigan, PhD., is Head of the Cardiovascular Infection Research Group and Principal Investigator in the Tissue Engineering Research Group at the Royal College of Surgeons in Ireland. Professor Kerrigan obtained his Ph.D. in infectious diseases from the Royal College of Surgeons in Ireland in 2001 and carried out postdoctoral research at the University of California, San Francisco. Professor Kerrigan's main research interests are developing and investigating host-microbe interactions in both 2D and 3D *ex vivo* model systems of bloodstream infections (bacteremia and sepsis) and elucidating the mechanisms that lead to metastatic spread to distant sites, such as the bone. Professor Kerrigan's research focuses primarily on the opportunistic pathogens *Staphylococcus aureus* and *Escherichia coli*.