



Skeletal infections: microbial pathogenesis, immunity and clinical management

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Abstract | Osteomyelitis remains one of the greatest risks in orthopaedic surgery. Although many organisms are linked to skeletal infections, *Staphylococcus aureus* remains the most prevalent and devastating causative pathogen. Important discoveries have uncovered novel mechanisms of *S. aureus* pathogenesis and persistence within bone tissue, including implant-associated biofilms, abscesses and invasion of the osteocyte lacuno-canalicular network. However, little clinical progress has been made in the prevention and eradication of skeletal infection as treatment algorithms and outcomes have only incrementally changed over the past half century. In this Review, we discuss the mechanisms of persistence and immune evasion in *S. aureus* infection of the skeletal system as well as features of other osteomyelitis-causing pathogens in implant-associated and native bone infections. We also describe how the host fails to eradicate bacterial bone infections, and how this new information may lead to the development of novel interventions. Finally, we discuss the clinical management of skeletal infection, including osteomyelitis classification and strategies to treat skeletal infections with emerging technologies that could translate to the clinic in the future.

Osteomyelitis is an infection of bone that can result from contiguous spread from surrounding tissue, direct bone trauma due to surgery or injury, or haematogenous spread from systemic bacteraemia. It remains a significant health-care burden with a prevalence of ~22 cases per 100,000 person-years in the United States, and its incidence has been rising over time, especially in the elderly and individuals with diabetes¹. Although it is a heterogeneous disease, subset classifications include implant-associated osteomyelitis (including peri-prosthetic joint infection (PJI) and instrumented spinal infections), fracture-related infection, acute haematogenous osteomyelitis, diabetic foot infection, septic arthritis and native spinal osteomyelitis.

Crucial to expanding our understanding of osteomyelitis and advancing treatment algorithms has been the application of animal models, which illustrate the interaction between the pathogen and cells of both the immune and skeletal systems in a manner that in vitro models cannot yet replicate. Animal models are available to study virtually all aspects of skeletal infection, and typically involve inoculation of bacteria at the time of implant placement (FIG. 1). They can vary in complexity from simple models where metal implants are placed under the skin (for example, tissue cage²) or into cortical

bone (for example, metal wire³) versus more complex models that mimic functional orthopaedic devices⁴. Additionally, approaches have been developed to induce non-implant infections by haematogenous inoculation into the tail vein⁵, direct inoculation into vertebral bodies or intervertebral discs⁶ to induce vertebral osteomyelitis, or inoculation into the foot pad of diabetic obese rodents to induce diabetic foot infection⁷.

As disease pathogenesis differs across different infection classes, so does microbial aetiology. Many different microorganisms have been implicated in skeletal infection, and the most common, along with their incidence and tropism, are shown in TABLE 1. In general, *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS), such as *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*, are responsible for up to two-thirds of all skeletal infections, with *S. aureus* being the most prevalent single pathogen. Additionally, antimicrobial resistance remains a challenge in osteomyelitis treatment with up to 50% of cases of *S. aureus* osteomyelitis caused by methicillin-resistant *S. aureus* (MRSA) strains⁸. Other less commonly identified pathogens include *Enterococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli* and *Cutibacterium acnes* (TABLE 1). Most cases of osteomyelitis are monomicrobial; however,

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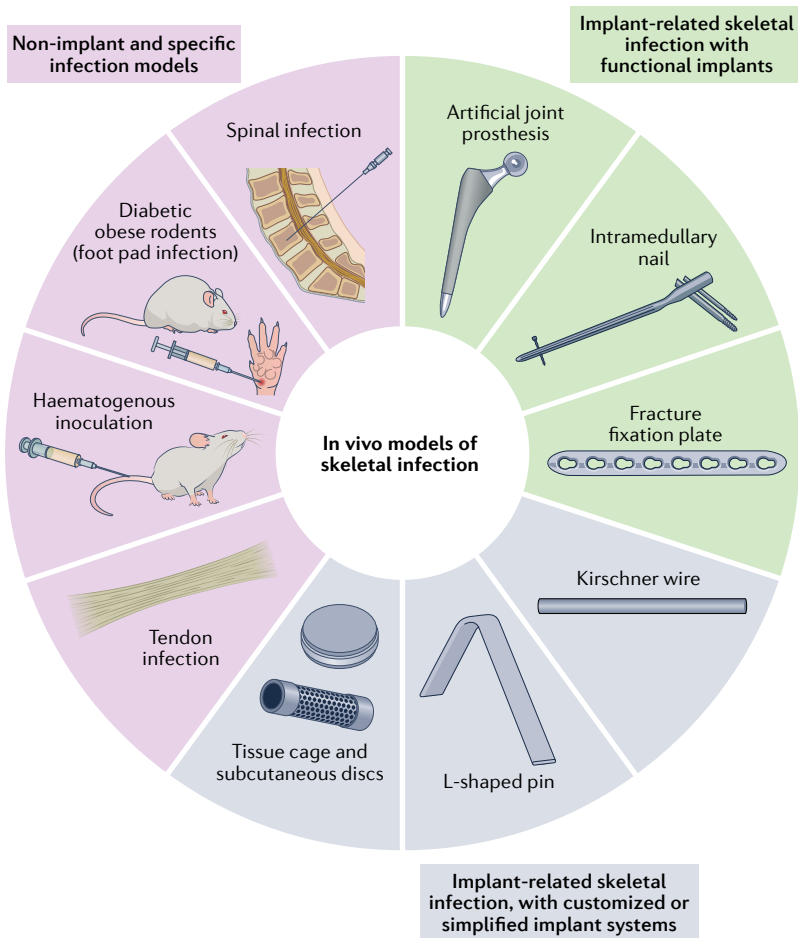


Fig. 1 | In vivo models of skeletal infection. Animal studies have been instrumental in some of the most important discoveries that have advanced our understanding of musculoskeletal infection. In broad terms, these models may be classified into implant-related or non-implant-related models. Many of the implant-related models utilize simplified biomaterials to represent the orthopaedic device (grey) that may be placed in the subcutaneous space (for example, tissue cages or discs) or within the bone (for example, Kirschner wires or pins) and offer simplicity and a relatively low risk profile. Certain studies may require use of functional devices whereby the implant placed in the animal serves to fix a fracture or replace a joint (green) and these may be particularly useful where bone healing, implant biomechanics or regulatory approval is the goal. Finally, several models have been developed that do not fit within either category as they may model specific disease entities (purple), such as haematogenous inoculation, leading to osteomyelitis, tendon infection, vertebral osteomyelitis or the use of comorbid mice such as diabetic obese mice.

Osteocyte lacuno-canalicular network (OLCN). Network of interconnected bone cells, called osteocytes, and their cellular processes contained in lacunae and canaliculi embedded within mineralized bone matrix.

Osteoblasts
Single nucleus bone cell responsible for the synthesis and mineralization of bone.

polymicrobial infections represent an important subset of cases in many different types of osteomyelitis, including trauma-related or fracture-related infections and infections of the foot or ankle.

In this Review, we summarize recently described mechanisms of *S. aureus* osteomyelitis pathogenesis, including intracellular infection, staphylococcal abscess communities (SACs) and invasion of the osteocyte lacuno-canalicular network (OLCN). We describe emerging mechanisms and pathophysiology of skeletal infections caused by other pathogens, including CoNS, *C. acnes* and *Streptococcus agalactiae*. Furthermore, we discuss various host immune evasion strategies employed by these pathogens during the establishment of osteomyelitis and introduce the theory of susceptible versus protective

immune proteomes in the context of skeletal infections. Finally, we summarize current clinical standards of care and controversies for the treatment of skeletal infections, challenges in diagnosing the causative pathogens, and emerging strategies in prevention and treatment.

S. aureus in skeletal infections

S. aureus is the most prevalent and most destructive pathogen in skeletal infections. From asymptomatic skin colonization to life-threatening disease, this Gram-positive bacterium has evolved to infect nearly every human tissue type. *S. aureus* is specifically pathogenic in skeletal infections because of its unique ability to invade, colonize and thrive within bone. Key mechanisms for *S. aureus* persistence in skeletal infections are outlined in FIG. 2.

Intracellular infection

The role of intracellular infection during osteomyelitis remains an active topic of research. Many groups have proposed long-term intracellular infection of bone cells by *S. aureus* as a mechanism for infection persistence and recurrence following long periods of dormancy, which is frequently observed in clinical cases of *S. aureus* osteomyelitis⁹.

S. aureus intracellular persistence has been described in a variety of cell types, including macrophages¹⁰, keratinocytes¹¹, epithelial cells¹² and endothelial cells¹³. Specifically, intracellular infection of macrophages, oftentimes called ‘Trojan horse’ macrophages, can facilitate bacterial dissemination through the body and enrichment for small colony variants (SCVs)¹⁰. *S. aureus* invasion of non-professional phagocytes has been described as host-mediated uptake where fibronectin bridges *S. aureus* cell-surface fibronectin-binding proteins A or B (FnBPA or FnBPB) with host cell $\alpha 5 \beta 1$ integrins, triggering cytoskeletal reorganization and bacterial cell uptake^{13,14}.

In the setting of bone, in vitro studies have shown *S. aureus* invasion and survival within osteoblasts^{15–17}, osteoclasts^{18,19} and osteocytes²⁰, and clinical case studies have described intracellular colonization of fibroblasts, osteoblasts and osteocytes from chronically infected bone tissue^{21,22}. Direct infection of bone cells is particularly pathogenic as it has been shown to induce the secretion of osteoclastogenic cytokines, contributing to pathological bone loss. Furthermore, some hypothesize that intracellularly infected osteoblasts that undergo differentiation or maturation into osteocytes could serve as a long-term and immune-privileged reservoir for bacterial colonization of bone²³.

Owing to limited in vivo studies, it is unclear how long *S. aureus* can persist inside bone cells, and how many bone cells are typically colonized with *S. aureus* in an infected lesion. Therefore, further studies using in vivo models of osteomyelitis are required to elucidate the involvement of *S. aureus* intracellular colonization in osteomyelitis.

OLCN invasion

The recent discovery of *S. aureus* invasion of the OLCN of cortical bone during osteomyelitis may provide another explanation for long-term bacterial persistence

Osteoclasts

Multi-nucleated bone cell responsible for the resorption of mineralized bone.

Osteocytes

Long-lived and most abundant bone cell, derived from osteoblasts and embedded within mineralized bone matrix.

Canaliculi

Microscopic canals within mineralized bone that contain the radiating cellular processes of osteocytes to form an interconnected network.

Durotaxis

Cell migration guided by rigidity or stiffness gradients of a substrate or extracellular matrix.

Haptotaxis

Cell migration guided by a gradient of adhesive substrates such as extracellular matrices.

and treatment failure in osteomyelitis. In contrast to the dogma that *S. aureus* is a non-motile cocci organism, recent studies in experimental models²⁴ and clinical cases²⁵ of osteomyelitis have revealed that *S. aureus* can invade and persist within the OLCN. Notably, *S. aureus* deforms to approximately half its native size to colonize the narrow confines of canaliculi, which are 100–600 nm in diameter²⁶. *S. aureus* cell wall synthesis machinery and surface adhesins may enable invasion of the OLCN guided by durotaxis²⁷ and haptotaxis²⁸. To test this hypothesis, an in vitro model, called the μ SiM-CA and designed to mimic canalicular invasion, was used to identify *S. aureus* genes required for canalicular invasion²⁹. In vitro and in vivo studies identified *pbp3* and *pbp4* as crucial genes for sub-micron propagation and invasion of the OLCN³⁰. *pbp3* and *pbp4* encode non-essential cell wall transpeptidases penicillin-binding proteins 3 and 4 (PBP3 and PBP4), which function in the final stages of cell wall synthesis³¹. This work suggests that inhibition of PBP3 and/or PBP4 may prevent *S. aureus* OLCN invasion, and their inhibitors could be used as adjuvants for antimicrobial treatment of *S. aureus* osteomyelitis.

Currently, it is unclear whether antibiotics readily diffuse into the OLCN of cortical bone at inhibitory concentrations. Murine studies have shown that the small molecule bromodeoxyuridine diffuses into infected canaliculi in vivo when added to the animal's drinking water²⁴. However, vancomycin treatment fails to eradicate MRSA within the OLCN of infected bone³⁰, and gentamicin treatment induced cell wall thickening of *S. aureus* within canaliculi³². These results suggest that both parenteral and local high-dose antibiotics reach bacteria within the OLCN at sub-inhibitory concentrations, thereby inducing the tolerant or persistent phenotypes. Novel therapies designed to inhibit OLCN invasion may be an effective adjuvant in combination with other bactericidal agents to improve infection eradication and reduce bacterial persistence.

Biofilm formation

An important and well-studied mechanism for *S. aureus* pathogenesis in osteomyelitis is the formation of biofilms, which has been extensively reviewed^{33,34}. *S. aureus* biofilms on necrotic bone and implant surfaces are particularly hard to eradicate because they limit antibiotic diffusion to bacterial cells, inhibit immune cell penetration and resist mechanical disruption. Furthermore, bacterial cells within a biofilm are metabolically diverse due to the gradients of nutrient and oxygen availability, thereby facilitating the selection of SCVs and persister cell populations.

The Agr quorum-sensing system is a key regulator of *S. aureus* biofilm formation. At low cell densities, *S. aureus* expresses microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (for example, FnBPA, FnBPB, Cna and Spa), which initiate adhesion to an abiotic substrate³⁵. Following adhesion, the bacterial cells continue to proliferate, and synthesize and secrete extracellular polymeric substances such as polysaccharide intercellular adhesion and extracellular DNA, involving the expression of the *ica* operon³⁶. At high cell densities, autoinducing peptides reach a threshold concentration that activates the AgrC–AgrA two-component system³⁷. The activation of the Agr system results in downregulation of MSCRAMMs and upregulation of phenol-soluble modulins (PSMs) and secreted toxins (for example, α -haemolysin (Hla), Pantan–Valentine leukocidin (PVL) and toxic shock syndrome toxin 1 (TSST1)), which in turn triggers bacterial dispersal from the mature biofilm³⁵.

Abscess formation

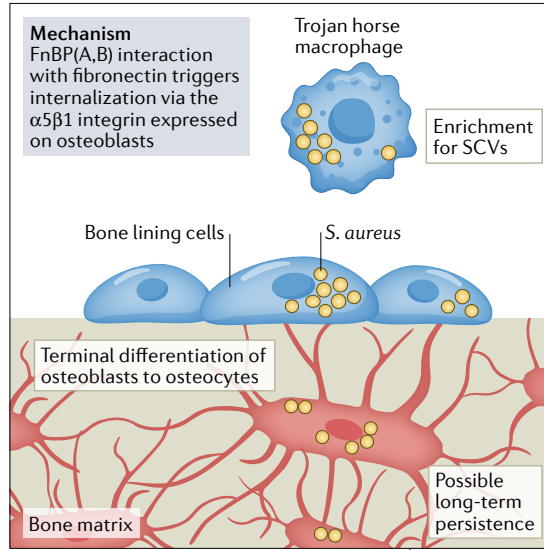
In addition to biofilms, *S. aureus* has the unique ability to chronically infect bone marrow and peri-implant soft tissue by forming robust SACs during osteomyelitis. The presence of SACs at the site of a bone infection is often used to diagnose and/or classify the stage of osteomyelitis³⁸ as SACs can greatly increase the severity

Table 1 | Causative pathogens and their incidence in skeletal infections

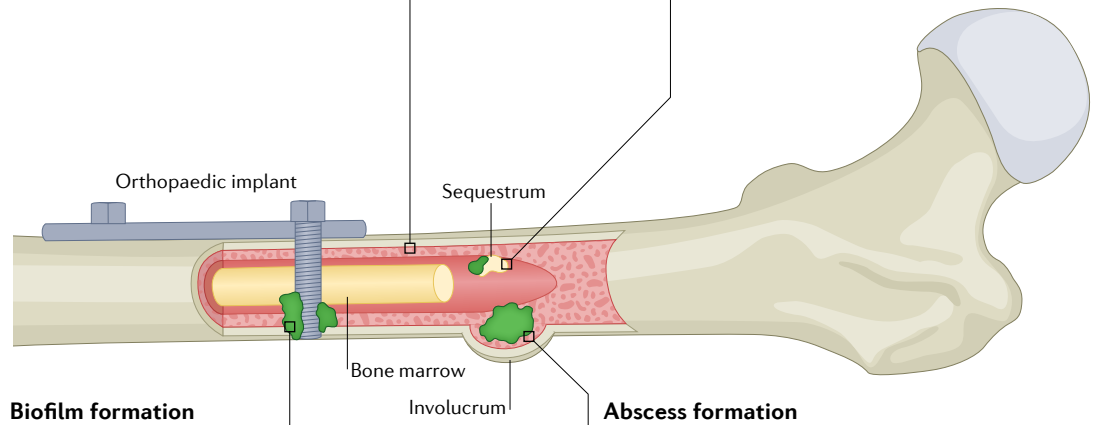
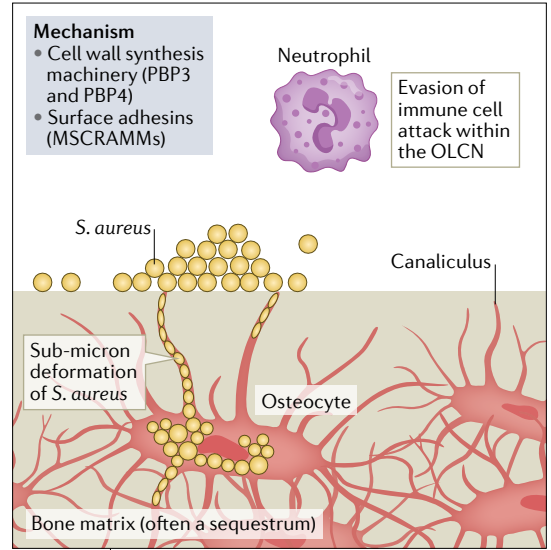
Species	Upper extremity ^{189,190}	Vertebral ^{191–193}	Lower extremity prosthetic joint ^{194–196}	Trauma or fracture related ^{8,197,198}	Haematogenous ¹⁹⁹	Foot and ankle ^{200,201}
<i>Staphylococcus aureus</i>	10–40	15–60	20–30	20–40	40	45–55
CoNS (<i>Staphylococcus epidermidis</i>)	10–20	5–40	25–35 (20–25) ^a	10–40 (5–15) ^a	<5	<5
<i>Streptococcus</i> spp.	5–10	5–10	<5	5–10	5–10	5–20
<i>Enterococcus</i> spp.	<5	5–15	<5	5	<5	5
Diphtheroids (<i>Cutibacterium acnes</i>)	<5 (30–50 in shoulder)	5 (10–15 post spinal fusion)	<5	5	<5	<5
Gram-negative	5–10	10–40	5–10	20	10–15	35–55
<i>Pseudomonas</i> spp.	<5	5–10	<5	5–10	5–10	10–20
<i>Enterobacteriaceae</i> spp.	<5	10–20	<5	5–20	5	10–15
HACEK	<5	<5	<5	<5	<5	<5
Fungal	<5	<5	<5	<5	<5	<5
Polymicrobial	10–25	15–30	10–20	20–30	20	30–80

Numbers reflect incidence range in percentage. CoNS, coagulase-negative staphylococci; HACEK, *Haemophilus* spp., *Aggregatibacter* spp., *Cardiobacterium* spp., *Eikenella* spp., *Kingella* spp. ^aNumbers in parentheses represent the incidence of *Staphylococcus epidermidis* within the CoNS group.

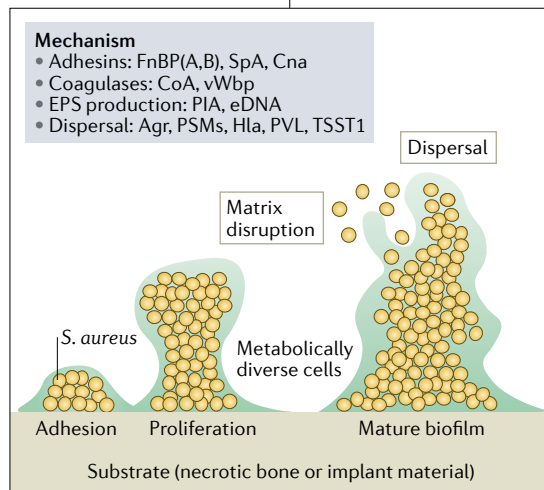
Intracellular infection



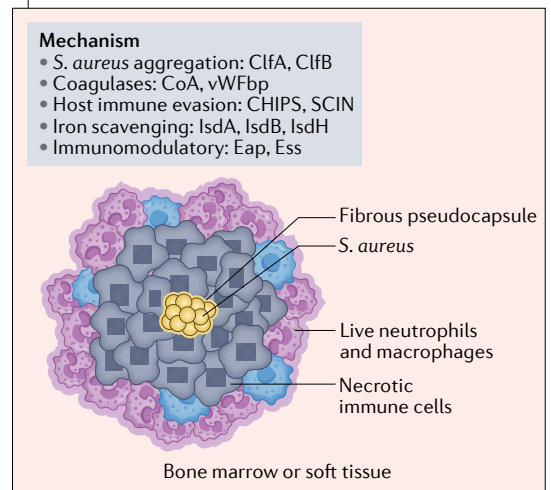
OLCN invasion



Biofilm formation



Abscess formation



of infections by restricting blood flow to the area³⁹. Although these bacterial lesions, also described as Brodie abscesses when found in bone tissue, have been studied for two centuries⁴⁰, recent research has formally demonstrated their resistance to standard-of-care antibiotics and host immune responses^{41,42}.

The formation of SACs represents an effective manipulation of the host response to prolonged *S. aureus* survival within soft tissues. SACs occur when bacteria

exploit the host response to encase themselves in a protective barrier and persist for prolonged periods of time⁴¹. *S. aureus* first enables the formation of a protective fibrous pseudocapsule through the activity of coagulase (CoA) and von Willebrand factor-binding protein (vWbp), which bind prothrombin to activate its conversion of fibrinogen into fibrin, and clumping factors A and B (ClfA and ClfB), which directly bind fibrinogen^{43,44}. Additionally, *S. aureus* secretes immune

◀ Fig. 2 | **Staphylococcus aureus pathogenesis in osteomyelitis.** *Staphylococcus aureus* employs a variety of pathogenic mechanisms during skeletal infection. Intracellular infection of osteoblasts, osteoclasts and osteocytes has been investigated as a possible source of long-term *S. aureus* persistence during osteomyelitis and ‘Trojan horse’ macrophages have been shown to cause bacterial dissemination and multiorgan failure. *S. aureus* invasion of the osteocyte-lacuno canalicular network (OLCN), most commonly within a sequestrum, permits evasion of host immune cells during osteomyelitis and requires *S. aureus* deformation to invade canaliculi of bone. *S. aureus* biofilms formed on implant surfaces and necrotic bone confer resistance to immune cell attack and antibiotic therapy through diffusion limitations and metabolic diversity of *S. aureus* cells. Lastly, staphylococcal abscess communities can form within the medullary cavity of long bones and in associated soft tissue. Gram-positive *S. aureus* cells are found at the centre of an abscess surrounded by a fibrous pseudocapsule encasing bacterial cells, followed by layers of dead and live immune cells. Note the formation of a necrotic bone fragment (sequestrum) and new bone formation (involucrum) during prolonged infection. CHIPS, chemotaxis inhibitory protein of *S. aureus*; ClfA/B, clumping factor A/B; CoA, coagulase; Eap, extracellular adherence protein; eDNA, extracellular DNA; Ess, ESAT-6 secretion system; FnBPA/B, fibronectin-binding protein A/B; Hla, α -haemolysin; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; PBP3/4, penicillin-binding protein 3/4; PIA, polysaccharide intercellular adhesin; PSM, phenol-soluble modulins; PVL, Pantón–Valentine leukocidin; SCIN, staphylococcal complement inhibitor; SCVs, small colony variants; TSST1, toxic shock syndrome toxin 1; vWbp, von Willebrand factor-binding protein.

evasion proteins, such as chemotaxis inhibitory protein of *S. aureus* (CHIPS) and staphylococcal complement inhibitor (SCIN), to counteract initial acute immune responses⁴⁵. As *S. aureus* cells begin to aggregate and establish a colony, large numbers of immune cells, mostly neutrophils, infiltrate the area and become necrotic as a result of direct and indirect killing⁴⁶. The layer of mostly necrotic host immune cells inadvertently creates an additional protective barrier, preventing newly recruited host immune cells from penetrating the abscess and killing the bacteria⁴¹.

Within a SAC, bacterial cells are largely devoid of high amounts of nutrients and oxygen; therefore, to survive in this environment, *S. aureus* must tailor its gene expression according to nutrient availability. One nutrient required for growth and proliferation of nearly all microorganisms is iron. *S. aureus* utilizes the iron-scavenging proteins IsdA, IsdB and IsdH to bind haemoglobin to extract haem as a source of iron^{47,48}. Extracellular adherence protein (Eap) and the ESAT6 secretion system (Ess) pathway, a non-canonical protein secretion pathway, have also been shown to promote SAC persistence particularly in the later stages of disease⁴⁷. Similar to biofilms, the cells within a SAC can be considered a metabolically diverse population contributing to antibiotic tolerance and resistance to immune cell attack. Ultimately, the only effective means of eradication is abscess disruption or debridement.

Persister cells and SCVs

Skeletal infections also involve bacteria existing as persister cells and as SCVs, which can arise due to environmental triggers such as oxidative stress, nutrient limitation, intracellular residence and low pH. SCVs can be present in all of the persistence mechanisms described above (intracellular infection, OLCN invasion, biofilms and abscesses), further contributing to infection severity and complicating treatment.

SCVs are characterized by small colony size, slow growth, and reduced virulence and have been described

in biopsies of patients with skeletal infection⁴⁹. The low virulence of these strains does not seem to reduce fitness to cause disease and may support evasion of host immune defences, although the precise mechanisms remain to be determined⁵⁰. By contrast, persister cells are a sub-set of any bacterial population characterized by their ability to survive high bactericidal antibiotic concentrations without exhibiting any antibiotic resistance mechanism⁵¹. In this case, persistence is believed to be a naturally occurring heterogeneity phenomenon within bacterial populations and induced by the (p)ppGpp alarmone-induced stringent response, the SOS response, and biofilm formation⁵². Both SCV and persister cells can contribute to failure of antibiotic therapy and recurrence of infection, and are at present not adequately addressed in current medical treatment options for patients with skeletal infections⁵².

Other pathogens in skeletal infections

Factors affecting infection

Non-staphylococcal pathogens are also capable of causing bone infection and may have increased importance in certain anatomical locations or patient populations (TABLE 1; FIG. 3). The route of infection is also important. For example, direct contamination of the surgical site is often caused by skin colonizers like CoNS^{53,54}, whereas haematogenous infection often involves pathogens well equipped to survive in the bloodstream such as *Streptococcus* spp. Furthermore, fracture-related infections can be caused by various environmental pathogens such as *Enterobacter* spp., *P. aeruginosa* or *Acinetobacter baumannii*, the latter commonly isolated from combat-related injuries.

The presence of an implant is another crucial factor for infection by less pathogenic or opportunistic organisms. For example, *S. epidermidis* accounts for up to 25% of PJI (TABLE 1; FIG. 3). Similar to *S. aureus*, it is a skin commensal organism and rarely causes invasive infection in immunocompetent hosts; however, it can serve as an opportunistic pathogen, particularly in the setting of a medical device⁵⁵.

C. acnes is the most prevalent bacterial pathogen in upper extremity infections resulting in osteomyelitis. *C. acnes* is a facultative anaerobic, Gram-positive bacillus that has been isolated in 5% of lower extremity PJI cases and over 50% of shoulder PJI cases^{56,57}. It is both a normal part of the microbiota of the skin, hair follicles, and sebaceous glands and an opportunistic pathogen in acne vulgaris and implant-associated osteomyelitis⁵⁸. *C. acnes* poses an additional challenge in the setting of implant-associated infection because clinical diagnostic criteria rely heavily on tissue culture results; however, this organism is difficult to culture, requiring repetitive testing and extended incubation times of up to 2 weeks⁵⁹. Similar to *S. epidermidis*, in vivo studies suggest that *C. acnes*-caused osteomyelitis is dependent on the presence of an implant, emphasizing the crucial role of biofilm formation in its pathogenesis⁶⁰.

As mentioned, certain microorganisms have a predilection for particular patient populations (FIG. 3). For example, *Salmonella* spp. are unique pathogens in the setting of haematogenous osteomyelitis in patients with

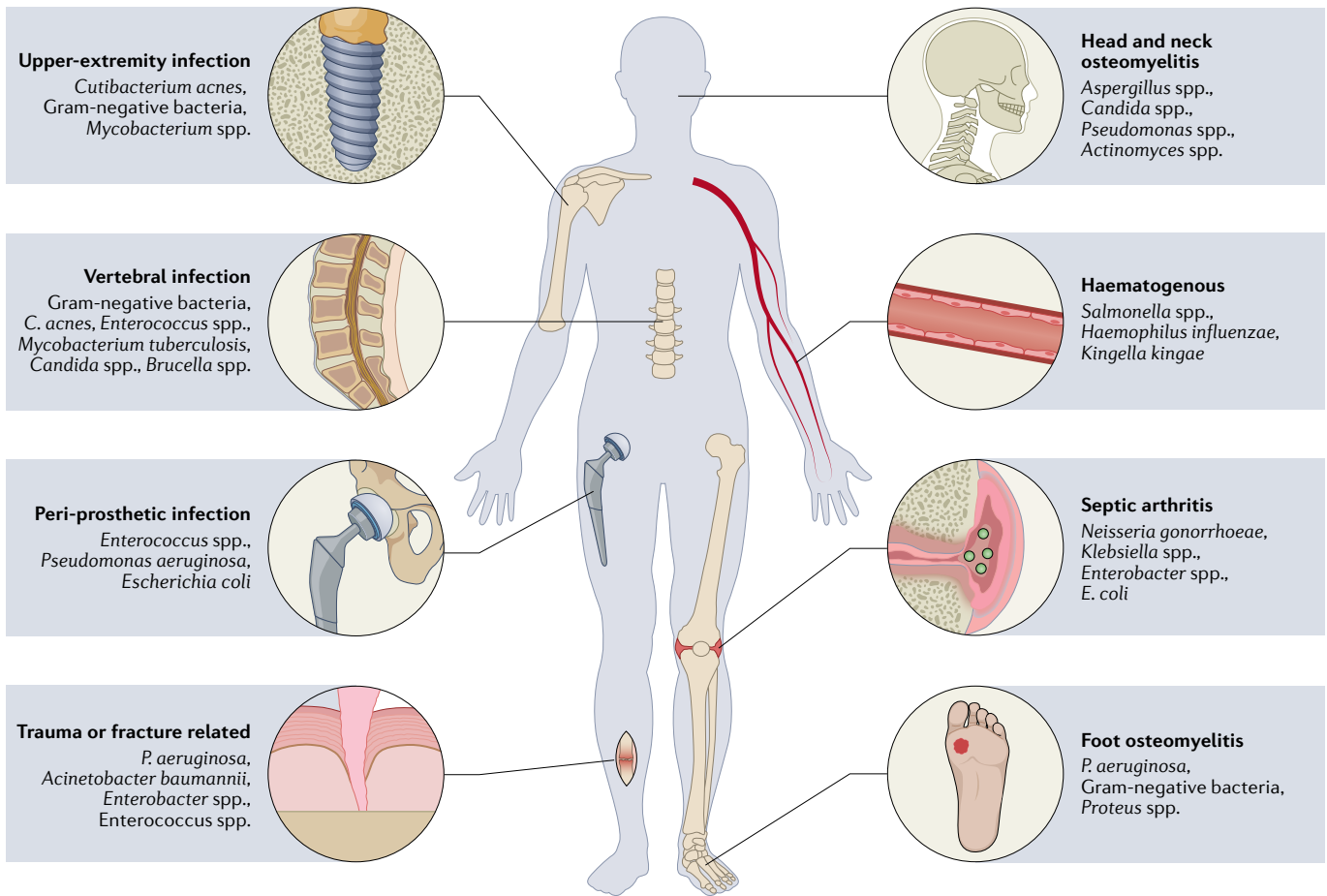


Fig. 3 | **Osteomyelitis pathogens beyond Staphylococcus spp. and Streptococcus spp.** Although *Staphylococcus* spp. are the most common pathogens across all skeletal infection types, other pathogens frequently cause infection within specific infection classes or patient populations. The figure highlights lesser common pathogens, beyond *Staphylococcus* spp. and *Streptococcus* spp., that are uniquely associated with skeletal infections across the human body. *Salmonella* spp. is most common in individuals with sickling haemoglobinopathies, *Haemophilus influenzae* and *Kingella kingae* are most common in children, and *Neisseria gonorrhoeae* is found in sexually active patients.

haemoglobinopathies, such as sickle cell anaemia, due to their functional asplenia and resulting deficient splenic clearance of opsonized encapsulated organisms⁶¹. In children, *Kingella kingae* has replaced *Haemophilus influenzae* as the predominant cause of septic arthritis since the advent of the *H. influenzae* vaccination. Gram-negative cocci, such as *Neisseria gonorrhoeae*, remain an important component of the differential diagnosis in septic arthritis in sexually active individuals⁶². Finally, in the setting of tissue ischaemia, such as the microvascular ischaemia of diabetic foot wounds, or in the setting of immunocompromised hosts, Gram-negative bacterial infections and polymicrobial infections become more prevalent. Although *Staphylococcus* spp. are the most prevalent pathogen across all infection types (TABLE 1), other pathogens can infect bone in specific circumstances and should be considered during disease diagnosis and treatment.

Pathogenesis

Despite being known as a less pathogenic species, *S. epidermidis* is commonly isolated from osteomyelitis cases involving an implant or an immunocompromised

host. Although most osteomyelitis studies have focused on *S. aureus*, opportunistic infection by the skin-colonizer *S. epidermidis* remains an important area of research. Similar to *S. aureus*, *S. epidermidis* adheres to implant surfaces via adhesins such as autolysin (AtlE), extracellular DNA, staphylococcal surface proteins 1 and 2, and cell wall-anchored proteins such as MSCRAMMs⁶⁵. *S. epidermidis* is capable of forming a robust biofilm on the implant surface, which contributes to immune evasion and antimicrobial resistance⁶³. However, *S. epidermidis* lacks the diversity of virulence factors expressed by *S. aureus* and, as a result, tends to manifest as a more indolent, less inflammatory infection⁶⁴.

Streptococcus spp. are the second most common bacteria isolated from implant-associated osteomyelitis, of which *S. agalactiae* is the most common species^{65–67}. Recent studies found that *S. agalactiae* infection induces significantly less osteolysis than *S. aureus*. Additionally, *S. agalactiae* infection showed decreased abscess formation compared with *S. aureus* and zero *S. agalactiae* colony forming units were recovered from the implant⁶⁸. These findings support the notion that *S. agalactiae* does not form robust implant-associated biofilms and suggest

Asplenia

The absence of a spleen, which can occur either as anatomical absence or functional absence.

Osteolysis

The process of bone destruction, typically as a pathological response to infection, inflammation or the presence of an implant.

a possible explanation for the improved efficacy of debridement and antibiotic and implant retention therapy observed in streptococcal infections. Additionally, *S. agalactiae* may preferentially colonize the vascular network within bone⁶⁸ as opposed to the OLCN invasion observed in *S. aureus* infection.

Indeed, many microbial pathogens beyond *Staphylococcus* spp. and *Streptococcus* spp. have been reported to form robust biofilms. For example, osteomyelitis-causing Gram-positive *Enterococcus* spp. have been reported to form biofilms in the setting of an implant material⁶⁹. Although the genetic determinants of *Enterococcus* spp. biofilm formation are not well understood, recent work has shown that *ahrC*, encoding a transcriptional regulator, and *eep*, encoding a membrane metalloprotease, contribute to *Enterococcus faecalis* biofilm formation⁷⁰.

Notably, Gram-negative *P. aeruginosa* is a notorious biofilm-forming bacterium known specifically for infections in the setting of cystic fibrosis and chronic wounds⁷¹. Although few studies have investigated *P. aeruginosa* biofilms specifically in the context of osteomyelitis, *P. aeruginosa* biofilms are known to contribute to antibiotic resistance and infection persistence, and should therefore be considered as an important mechanism of pathogenesis in osteomyelitis⁷².

Furthermore, multiple osteomyelitis-causing pathogens have been reported to form SCVs. In fact, the SCV phenotype was originally described for *Salmonella enterica*⁷³ and has since been reported in a range of osteomyelitis-causing pathogens, including *S. epidermidis*⁷⁴, *E. coli*⁷⁵ and *P. aeruginosa*⁷⁶. However, the prevalence of SCVs of pathogens beyond *S. aureus* in osteomyelitis is not well known and requires further research.

Polymicrobial infections

Polymicrobial infections are the cause of approximately one-third of post-traumatic osteomyelitis cases⁷⁷, 6–21% of PJI⁶⁵, and as high as 83% of diabetic foot infections⁷⁸. Unsurprisingly, the most prevalent organism in polymicrobial osteomyelitis is *S. aureus*, with co-infecting bacteria varying from opportunistic to pathogenic bacteria. In cases of infection site contamination (non-haematogenous), contamination by skin-colonizers commonly includes CoNS, whereas contamination by environmental organisms can be much more diverse, including *E. coli*, *Enterobacter* spp., *P. aeruginosa*, *A. baumannii*, *Bacillus* spp. and *Enterococcus* spp.

Oftentimes, polymicrobial infections are associated with poorer outcomes and increased infection severity⁷⁹. This can be explained by microbial synergism that induces specific virulence traits or modulates the host immune response⁸⁰. Interestingly, a study showed that *S. aureus* virulence is augmented by the addition of skin commensal organisms or peptidoglycan, acting as 'pro-infectious agents'⁸¹. On the other hand, commensal organisms are also known to play a protective role against pathogenic infection, supported by recent work from a study in which CoNS species, such as *Staphylococcus simulans*, inhibited *S. aureus* quorum sensing and reduced *S. aureus*-induced dermonecrosis in a murine model of skin infection⁸².

To expand our understanding of polymicrobial interactions in the setting of skeletal infection and to inform effective treatments, preclinical models of polymicrobial osteomyelitis are required. Currently, the number of studies investigating polymicrobial skeletal infection models are limited and not well established. Organisms studied in these models include *A. baumannii*, *E. coli* and *P. aeruginosa* in combination with *S. aureus*^{83–85}. In these models, *S. aureus* oftentimes outcompetes the co-infecting organism in the bone setting or on the implant material, resulting in fewer non-*S. aureus* colony forming units^{84,85}. This is explained in part by the arsenal of virulence mechanisms uniquely employed by *S. aureus* to thrive in the bone microenvironment.

More studies are required to understand specific microbial interactions that lead to increased severity in polymicrobial infections. One study showed that the presence of *P. aeruginosa* in bone tissue was increased during co-infection with *S. aureus* compared with *P. aeruginosa* monomicrobial infection⁸³, suggesting a role for microbial synergism in this model. However, the mechanism for *P. aeruginosa* and *S. aureus* synergism in skeletal infection is not yet understood.

Host responses and immune evasion

The immune system plays a crucial role in the defence against skeletal infections and maintaining bone homeostasis. This section describes the interaction between the immune system (innate and adaptive) and essential *S. aureus* virulence proteins during skeletal infections. In particular, immune activation, suppression and regulatory mechanisms will be discussed during chronic implant-associated osteomyelitis.

Tissue-specific immune responses

The ability of *S. aureus* to survive long-term in the bone niche is attributed to the expression of an array of virulence factors, including adhesins, immunomodulatory proteins, toxins and superantigens with redundant functions. FIGURE 4 illustrates the crucial proteins utilized by *S. aureus* to manipulate innate and adaptive host immune responses.

S. aureus-mediated manipulation of innate immune cells

Innate immune cells, including macrophages and neutrophils, provide the first line of defence against *S. aureus* skeletal infections. FIGURE 4a depicts important virulence proteins and mechanisms employed by *S. aureus* to evade innate immune cells.

Planktonic *S. aureus* cells are effectively killed by innate immune cells through phagocytosis, production of antimicrobial peptides, oxidative bursts, secretion of pro-inflammatory cytokines and chemokines, and formation of neutrophil extracellular traps, which trap bacteria^{86,87}. However, in chronic skeletal infections, such as implant-associated osteomyelitis, *S. aureus* persists as biofilms, leading to decreased neutrophil activity against *S. aureus*⁸⁸. *S. aureus* also secretes pore-forming toxins (for example, Hla, β -haemolysins, γ -haemolysins (HlgAB and HlgCB), leukocidin A/B (LukAB) and PVL) that directly kill neutrophils, macrophages and other antigen-presenting cells by damaging cellular membranes^{89,90}.

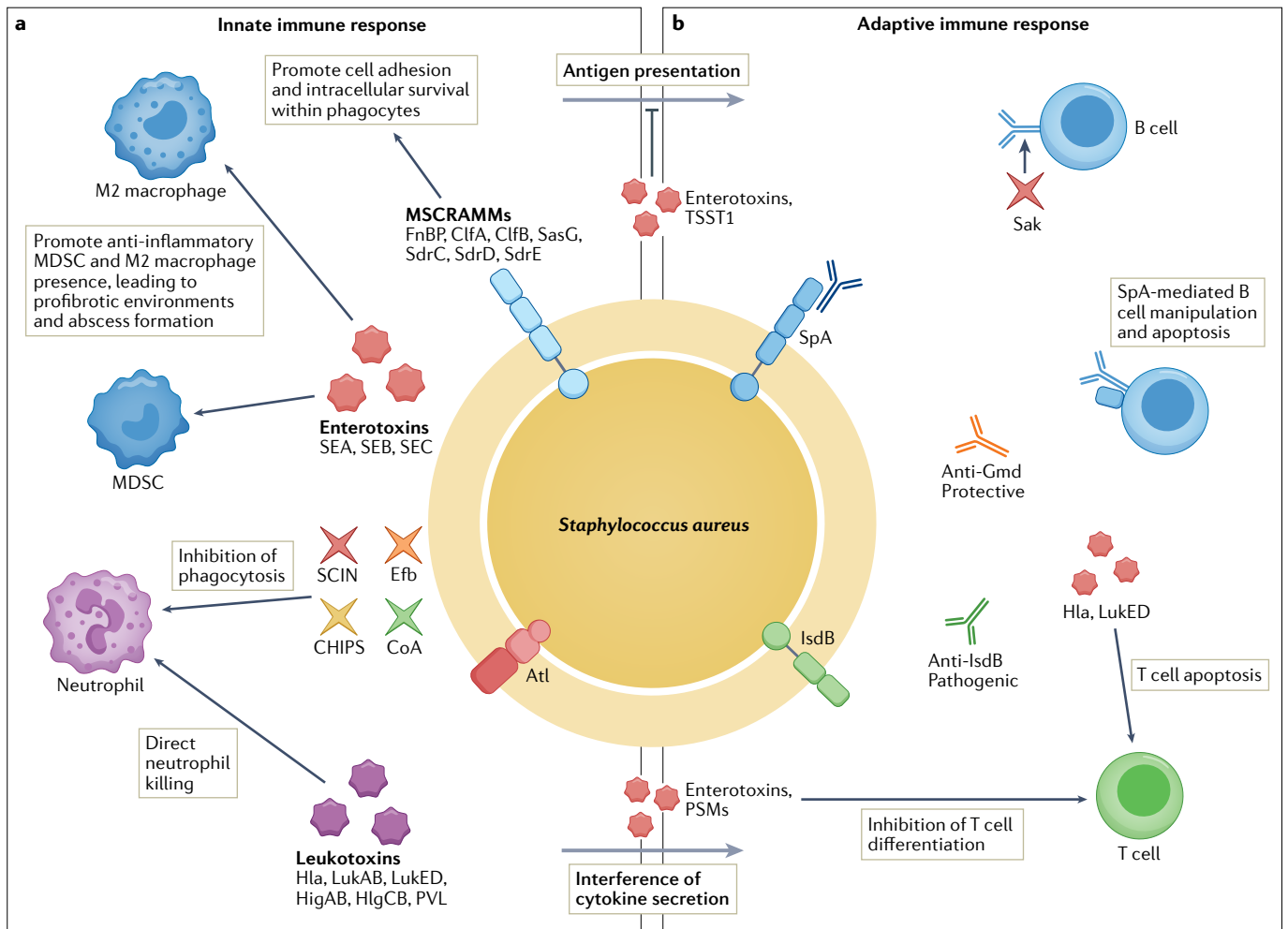


Fig. 4 | Innate versus adaptive immune responses to *Staphylococcus aureus* infections. Virulence proteins enable *Staphylococcus aureus* to successfully evade host immune responses. **a** | *S. aureus* utilizes several cell-associated microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and secretory proteins to thwart innate host defences. The bacterial cell surface protein SasG promotes cell adhesion, intracellular survival within neutrophils, macrophages and non-professional phagocytes. *S. aureus* impedes complement-mediated opsonization and phagocytosis by trapping complement proteins using extracellular fibronectin-binding protein (Efb), SpA and Sbi; inhibiting neutrophil recruitment using staphylococcal complement inhibitor (SCIN), chemotaxis inhibitory protein of *S. aureus* (CHIPS), SpA and coagulase (CoA); and secreting pore-forming toxins (α -haemolysin (Hla), β -haemolysin, γ -haemolysin (HlgAB, HlgCB), leukocidin AB (LukAB) and Panton–Valentine leukocidin (PVL)) to disrupt host membranes to kill innate cells. Superantigens (*S. aureus* enterotoxin B and C (SEB, SEC) and toxic shock syndrome toxin 1

(TSST1)) contribute to immune evasion by 1) skewing M2-macrophage polarization, 2) promoting myeloid-derived suppressor cell (MDSC) formation, leading to decreased phagocytosis, 3) promoting staphylococcal abscess community formation, and 4) contributing to dysregulation of antigen presentation and cytokine production that affects T and B cell activation. **b** | On the adaptive immunity side, superantigens can crosslink T cell receptors on tissue-resident T cells to cause antigen-independent stimulation, ultimately causing cell exhaustion. Hla, LukED and phenol soluble modulins (PSMs) can interfere with T cell differentiation and activation, and cause apoptosis. The multifunctional SpA protein can impede B cell function and staphylokinase (Sak) can cleave IgG to prevent antibody-mediated phagocytosis. Antibodies can either be protective or pathogenic during *S. aureus* infection, with anti-IsdB antibodies facilitating *S. aureus* survival within 'Trojan horse' macrophages causing sepsis, and anti-Gmd antibodies preventing biofilm formation and protecting against skeletal infections. AtI, autolysin; ClfA/B, clumping factor A/B; FnBP, fibronectin-binding protein.

Interestingly, *S. aureus* persists as a biofilm in the bone niche during chronic osteomyelitis by downregulating these toxins, especially Hla and LukAB^{91,92}. In skeletal infections, *S. aureus* biofilms can actively evade toll-like receptor 2 (TLR2) and TLR9 recognition due to bacterial ligand inaccessibility and skew macrophage responses towards an anti-inflammatory or profibrotic M2 phenotype⁹³. A typical M2 macrophage response is characterized by attenuated antimicrobial peptide production, increased arginase 1, IL-4 and IL-10 expression, and decreased inducible nitric oxide

synthase (iNOS) expression^{93,94}, all of which could lead to profibrotic responses creating abscesses during chronic implant-associated skeletal infections. Additionally, *S. aureus* also modulates the expression of myeloid-derived suppressor cells (MDSCs), which are a heterogeneous subset of immature monocytes and granulocytes⁹⁵. In a murine osteomyelitis model, one study showed that MDSCs actively suppressed pro-inflammatory cytokine production and T cell recruitment to the infection site, thus enabling bacterial persistence⁹⁶. It was also demonstrated that superantigens, such as staphylococcal

enterotoxins, play an important role in modulating MDSC generation, which affects bacterial persistence⁹⁷.

Additionally, *S. aureus* can survive within phagocytic cells and in non-professional phagocytes such as osteocytes and osteoblasts in the bone niche during chronic osteomyelitis^{17,20,98}. The internalization process within these cells is achieved by MSCRAMMs FnBPA and FnBPB binding to fibrinogen, and connecting to $\alpha 5\beta 1$ integrins on macrophages or neutrophils¹³. In addition, other MSCRAMMs, such as ClfA, ClfB, surface-anchored proteins (SdrC, SdrD and SdrE) and the surface protein SasG, promote bacterial aggregation and biofilm formation on both indwelling prosthetic devices and plasma-coated biological surfaces^{99,100}. The role of these adhesion proteins pertaining to *S. aureus* immune evasion and pathogenesis has been described elsewhere¹⁰⁰. In addition to intracellular survival in phagocytes, *S. aureus* also impedes complement-mediated opsonization and phagocytosis through secretion of virulence proteins such as CHIPS, SCIN, CoA and extracellular fibrinogen binding protein (Efb)^{54,101}.

***S. aureus*-mediated manipulation of adaptive immune cells.** Adaptive immunity against *S. aureus* encompasses cell-mediated T cell responses and humoral antibody responses mediated by B cells. FIGURE 4b illustrates the mechanisms and related immunomodulatory proteins utilized by this pathogen to evade adaptive immune cells.

Research over the past two decades has accumulated evidence that T cells and its subsets are essential to host defence against *S. aureus*¹⁰². Murine osteomyelitis models have shown that *S. aureus* biofilms skew CD4⁺ T helper (T_H) cell responses towards T_H1 and T_H17 cells, which causes ineffective clearance of intracellular pathogens¹⁰³. In contrast to these studies, one study found minimal T cell infiltration to the biofilm infection site in human *S. aureus* PJI¹⁰⁴. These studies highlight that the immune correlates of infection and protection in mice often diverge from human responses. A small animal model with a functional human immune system could attempt to bridge this gap. It was recently demonstrated that humanized NSG (NOD scid gamma) mice suffered exacerbated *S. aureus* osteomyelitis compared with normal C57BL/6J mice and an increased susceptibility to *S. aureus* osteomyelitis-induced sepsis. Interestingly, the authors observed infiltration and proliferation of human CD3⁺T-bet⁺ cells adjacent to SACs in the bone marrow of infected humanized NSG mice¹⁰⁵.

The aforementioned conventional T cell responses require *S. aureus* antigen presentation and interaction with T cell receptors (TCRs). However, superantigens such as TSST1 can crosslink the V β chain of TCRs from tissue-resident T cells, causing antigen-independent T cell stimulation and massive secretion of several cytokines¹⁰⁶. One study posited that such strong T cell activation could ultimately lead to impaired memory T cell responses and anergy¹⁰⁷. Nonetheless, the consequence of superantigen expression and its influence on adaptive immunity needs to be fully elucidated. Other pore-forming toxins, such as Hla and PSMs, can interfere with T cell differentiation and activation and also cause T cell apoptosis^{89,108,109}. In addition, *S. aureus*

can manipulate B cell survival and function and influence humoral antibody responses. It also secretes the multifunctional protein SpA, which binds to the Fc γ and Fab domains of certain antibodies^{110–112}, blocking antibody-mediated phagocytosis and concurrently initiating proliferative B cell apoptosis^{113,114}. Additionally, the *S. aureus* enzyme staphylokinase (Sak) can directly degrade IgG. Sak can strongly activate human plasminogen on cell surfaces, leading to cleavage and degradation of IgG¹¹⁵. The importance of humoral responses and anti-*S. aureus* antibody-mediated protective immunity is discussed in the subsequent section.

Protective versus susceptible immune responses to *S. aureus*

All humans develop anti-*S. aureus* antibodies due to life-long exposure to *S. aureus* through nasal carriage or prior infections. However, the presence of antibodies does not confer protection against future infections and the antibody repertoire against *S. aureus*, especially in healthy individuals, is highly variable¹¹⁶. Elucidating pathogen-specific antibodies to *S. aureus* and understanding the functional role of the protective versus susceptible nature of an individual's antibody response is essential for the development of successful immunotherapies against *S. aureus*. Recent studies have identified pathogenic antibody responses against haem-scavenging IsdB during *S. aureus* osteomyelitis in mice and humans, leading to susceptible host immunity^{117,118}. Surprisingly, patients from an international biospecimen registry¹¹⁹ with high circulating anti-IsdB antibody titres were more likely to have adverse outcomes (arthrodesis, reinfection, amputation and sepsis leading to death) from bone infection¹¹⁸. Interestingly, utilizing preclinical mouse models, it was demonstrated that anti-IsdB antibodies facilitate *S. aureus* internalization in 'Trojan horse' macrophages¹²⁰, followed by dissemination to the bloodstream and internal organs^{34,121}. Indeed, large amounts of 'Trojan horse' leukocytes were observed in patients that succumbed to *S. aureus* osteomyelitis-induced sepsis and death³⁴. Perhaps these mechanisms explain the failure of a 8,000-person cohort phase II/III clinical trial of an IsdB active vaccine (Merck V710), where individuals in the vaccinated group had a fivefold increase in mortality induced by multiple organ sepsis¹²².

In contrast to anti-IsdB antibodies, antibodies to the glucosaminidase (Gmd) subunit of *S. aureus* autolysin (Atl) can elicit protection against *S. aureus* osteomyelitis in mice^{123,124}. Most interestingly, high levels of anti-Gmd antibodies in patients correlated with a marked reduction in adverse outcomes due to *S. aureus* infection¹²⁵, suggesting a protective response. In agreement with the protective humoral immune proteome hypothesis, patients with high IgG concentrations against other *S. aureus* antigens, such as amidase (Amd), IsdH, CHIPS, SCIN and Hla, had a significant reduction in adverse outcomes¹¹⁸. The protective potential of anti-*S. aureus* antibody responses against toxins and superantigens has also been explored^{126,127}. In particular, the lack of anti-TSST1 antibodies correlated with increased risks of *S. aureus* toxic shock syndrome¹⁰⁶. Collectively, these studies make a strong case for prophylactic vaccines

Anergy

The lack of reaction by an immune cell, where they are functionally inactivated following an antigen encounter.

Arthrodesis

Surgical immobilization of a joint by fusion of the adjacent bones (also called joint fusion).

Sequestrum

A fragment of necrotic bone, separated from healthy bone tissue.

Synovial fluid

Viscous liquid within the cavities of synovial joints, which lubricates joints for articulation.

that can reverse this susceptibility towards a protective immune proteome before elective orthopaedic surgery.

Immune responses to other pathogens

CoNS are second only to *S. aureus* as the most prevalent species encountered in skeletal infections involving implants, although the clinical presentation is often milder and more chronic in nature. The lack of virulence factors, such as immunotoxins or immune-evasion mechanisms, in *S. epidermidis* compared with *S. aureus* may at least partially explain this observation and may be responsible for the distinctly different immune responses to both pathogens. As is common amongst bacterial species, *S. epidermidis* triggers immune responses partly via TLR2 (REF.¹²⁸) or NOD-like receptor recognition of cell wall molecules, including lipoproteins, lipoteichoic acid and peptidoglycan^{128–130}. *S. epidermidis* also has a number of secreted factors, such as PSMs, that activate the human immune system¹³¹. However, the immune response to *S. epidermidis* leads to markedly higher induction of the anti-inflammatory cytokine IL-10, for example, during skin colonization, than *S. aureus*¹³², which matches the clinical picture of *S. epidermidis* as a persistent but not aggressive pathogen in skeletal infections. The immune response to other pathogens has been less well studied. *C. acnes* has been extensively studied in the context of acne, where it has been shown to activate TLR2 and TLR4, leading to activation of the NF- κ B and MAPK signalling pathways and ultimately resulting in production of pro-inflammatory molecules such as IL-1 α , IL-1 β and IL-6 (REFS^{133,134}). Further investigations into *C. acnes* host–pathogen interactions are required to provide a more complete understanding of the key virulence factors for skeletal infection and mechanisms responsible for the relatively moderate osteolysis commonly associated with this microorganism.

Clinical management of skeletal infections

Classification of osteomyelitis

Classification of osteomyelitis remains controversial and many different classification systems have been used to better describe the spectrum of disease³⁹. Clinically, one of the most common ways to classify osteomyelitis has traditionally been based on temporal definitions^{135–137}, such as acute versus chronic infection, with the goal of defining the need for less aggressive (antibiotics alone) versus surgical treatments. It is now known that temporal thresholds do not exist, and clinical studies have used a wide range of temporal definitions from 1–2 weeks to 3–6 months¹³⁸. The formation of biofilms or OLCN invasion is likely the pathological event that defines a recalcitrant infection that may need more aggressive treatment; however, a temporal definition for these events is lacking. Common characteristics used to classify osteomyelitis include location and/or extent of bone involvement, the extent of necessary bone resection, presence of abscesses or sequestrum, aetiology of infection (haematogenous versus contiguous spread, for example), or need for soft tissue coverage during surgery^{135,137,139,140}. Another important variable that may help predict outcomes of osteomyelitis treatment is the immune status of the host and whether they are capable of withstanding surgical treatment^{139–141}.

One of the most common and well-accepted staging systems that is used is the Cierny–Mader classification, which tries to define the area of bone involvement (medullary — type I; superficial — type II; localized — type III; and diffuse — type IV) and the physiological class of the host (A — immunocompetent; B — locally or systemically compromised; and C — a host that will not tolerate surgery)¹⁴¹. This emphasizes the disease extent and the overall health of the affected patient, which are likely to be associated with treatment outcome.

Diagnosis, prevention and treatment

Osteomyelitis is a heterogeneous disease depending on the anatomical site, presence of an implant, and host–pathogen characteristics; however, some general principles in diagnosis and treatment can be defined. The standard approach to a patient with suspected osteomyelitis is summarized in BOX 1. Recent advances in the diagnosis of osteomyelitis include (1) standardization of diagnostic criteria; (2) the emergence of new biomarkers of infection; and (3) improvements in methods for organism identification. In recent years, national and international groups of experts have attempted to provide evidence-based, standardized reporting of musculoskeletal infection diagnosis and treatment (BOX 1). These are important first steps in improving communication between clinicians and providing a standard for clinical studies reporting. Serum and synovial fluid tests are frequently used as an initial screening in suspected cases of osteomyelitis (BOX 1). In recent years, the identification of novel biomarkers has provided the potential to improve diagnostic accuracy. For example, α -defensin, which is an antimicrobial peptide released mostly by activated neutrophils, has become a novel synovial fluid biomarker for the diagnosis of PJI¹⁴². In cases of suspected osteomyelitis, advanced imaging studies, such as magnetic resonance imaging, or nuclear medicine studies, such as technetium-99m bone scans, are typically used to identify the anatomical location of osteomyelitis and the presence of associated abscesses and to assist with obtaining image-guided tissue biopsy specimens (BOX 1). Most diagnostic and treatment criteria rely on the identification of the infectious organism; however, culture-negative infection can represent up to 20% of cases in osteomyelitis, creating diagnostic and treatment uncertainty. Novel methods to improve organism identification include matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS), genomic sequencing, and implant sonication. MALDI–TOF MS analysis enables the identification of bacterial species from colonies directly from existing media or blood culture, which was a significant improvement in time, labour and cost compared with prior methods¹⁴³. Given the concomitant existence of implant-associated biofilms in many types of osteomyelitis, direct cultures of fluid after sonication of the retrieved implant is another way to improve the sensitivity of organism identification¹⁴⁴. Genomic sequencing-based methods have been an important area of investigation in recent years in diagnosing skeletal infections. Next-generation sequencing (NGS)-based methods involve both targeted or

Box 1 | Clinical guidelines for acute and chronic osteomyelitis

Diagnosis

- Physical exam findings: fever, constitutional symptoms, sinus tract, skin erythema, joint pain
- Serum or joint fluid laboratory testing: erythrocyte sedimentation rate, c-reactive protein (CRP), peripheral blood cultures, serum white blood cell count, α -defensin, D-dimer, synovial IL-6, synovial CRP
- Site-directed radiological imaging: plain radiographs, CT scan, magnetic resonance imaging, bone scan, nuclear medicine studies
- Tissue culture: image-guided bone biopsy, surgical tissue retrieval, synovial fluid aspiration

Treatment

- Pathogen-directed systemic antimicrobial therapy: intravenous versus oral
 - Pathogen-directed oral antibiotics with appropriate bioavailability may be non-inferior to intravenous therapy for treatment of chronic osteomyelitis²⁰²
- Pathogen-directed systemic (oral and intravenous) antimicrobial therapy: duration of treatment
 - Traditional minimum 4–6 weeks antibiotic therapy
 - Shorter durations may be acceptable in children
 - Immunosuppression, including diabetes and chronic osteomyelitis, may need longer durations (6–12 weeks)^{203,204}
 - Long duration (3+ months) oral antibiotic suppression may improve outcomes in the setting of an implant
- Surgical debridement of affected region
 - Required in implant-associated chronic osteomyelitis, soft tissue or bone abscess
 - May not be necessary for haematogenous osteomyelitis in children
- Implant retention versus removal
 - Implant retention can lead to decreased success rates during surgical irrigation and debridement
 - Implant removal, however, can increase patient morbidity and may not be possible in certain circumstances where bone or joint stability is required, or where implant removal may cause unacceptable patient morbidity (fracture-associated, spinal implants or joint replacement implants)
- Local antimicrobial therapy
 - Antibiotic-containing cement, topical antibiotic powder, antibiotic beads may have some benefit in certain cases of chronic osteomyelitis but considerable controversy still exists
 - Indicated in setting of implant removal when temporary internal fixation necessary for bone stability

Examples of evidence-based clinical guidelines

- Clinical practice guideline for the diagnosis and treatment of acute haematogenous osteomyelitis in children developed by the Paediatric Infectious Diseases Society (PIDS) and the Infectious Diseases Society of America (IDSA)²⁰⁵
- Fracture Related Infection Consensus Group (2020)²⁰⁶
- International Working Group on the Diabetic Foot (2019)²⁰⁷
- Proceedings of the Second International Consensus Meeting on Musculoskeletal Infection (2018)^{138,176,208–210}
- European Society for Paediatric Infectious Disease (ESPID) Bone and Joint Infection Guidelines (ESPID Guidelines)²¹¹
- IDSA Clinical Practice Guidelines for the Diagnosis and Treatment of Native Vertebral Osteomyelitis in Adults (2015)²¹²
- Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection (2013)^{213,214}

untargeted massive parallel DNA sequencing to identify both human and microbiological nucleic acids within a sample. Untargeted methods of NGS, such as metagenomic sequencing, have been shown to be accurate in identifying known pathogens from culture-positive PJIs (95% concordance with sonicate fluid culture) while also identifying organisms in over 40% of culture-negative

infections that otherwise met diagnostic criteria for infection¹⁴⁵. Concerns about genomic sequencing-based technology include cost and the identification of colonizing versus actively infectious organisms. For example, presumed aseptic primary joint replacements with no clinical signs of infection identified an infectious organism in 35% of cases using NGS, suggesting that further refinement is necessary to identify active infections¹⁴⁶.

Infection prevention is another crucial method to reduce the impact of osteomyelitis. Systemic antibiotic therapy prior to surgical incision in implant-associated surgery is one of the most effective evidence-based preventive therapies, and forms part of the Surgical Care Improvement Project national guidelines on infection prevention¹⁴⁷. However, the use of local antibiotics, including antibiotic powder, beads and antibiotic-eluting cement, for infection prevention has yielded mixed results. For example, certain combinations of antibiotic-eluting cement may decrease the risk of PJI in high-risk patient populations such as femoral neck fractures, veteran patients and paediatric spinal deformity surgery^{148–150}. However, other studies have shown no benefit to infection prophylaxis and poor cost-effectiveness for commercially available antibiotic bone cement formulations when used across a broad population^{151–153}. One strategy for infection prevention is active immunization. Unfortunately, vaccine strategies targeting components of the *S. aureus* cell wall, such as poly-*N*-acetyl glucosamine, lipoteichoic acid, capsular polysaccharides or iron-acquisition such as IsdB, have been unsuccessful beyond phase I clinical trials^{122,154,155}. Newer vaccine strategies targeting virulence factors (Hla and PVL) or multivalent vaccines targeting capsular or surface antigens have not progressed beyond early-stage trials^{156,157}. Another promising early-stage systemic treatment in osteomyelitis is the use of immunotherapy. For example, monoclonal antibodies targeting Hla, biocomponent cytotoxins and ClfA showed a significant reduction in infection severity using a rabbit model of *S. aureus* PJI¹⁵⁸.

Establishing both local and systemic infection control are crucial principles to treating osteomyelitis in adults. Surgical debridement and systemic antibiotics are required in most adult patients with osteomyelitis. The overall success of surgical treatment varies widely and depends on anatomical location, soft tissue integrity, presence or absence of an implant, presence of a deep abscess or biofilm, immune status of the host, and the infecting organism. For example, infection cure rates vary between 50–75% for debridement and implant retention in the setting of acute postoperative or haematogenous infection in lower extremity joint replacement and 60–90% for chronic infections treated with two-staged implant removal^{159–162}. For hospitalized patients with diabetic foot infections, either minor or major amputations are needed in up to 70% of cases¹⁶³. The infecting organism may influence outcomes, and *Staphylococcus* spp. may have worse outcomes with debridement and implant retention relative to other organisms in infected lower extremity joint replacement¹⁶⁴. In contrast to adults, children with haematogenous osteomyelitis may be treated with systemic antibiotics alone unless deep abscess or intra-articular involvement is present or in cases

with MRSA¹⁶⁵. In addition to surgical debridement, systemic antibiotic therapy is a crucial component to osteomyelitis treatment. Principles of systemic antibiotic therapy in osteomyelitis include organism-directed therapy based on sensitivities, use of agents that achieve appropriate osseous concentrations, and the use of anti-biofilm agents in the setting of a retained implant. Rifampicin is typically used as an anti-biofilm agent in implant-associated infections; however, rapid resistance can develop when used as a monotherapy and it is therefore used in combination with other systemic agents¹⁶⁶. One strategy to improve treatment in osteomyelitis is the development of novel antibiotics with higher oral bioavailability, broad spectrums of activity against resistant organisms and longer half-lives to shorten the necessary duration of treatment¹⁶⁷. One novel approach to target intracellular bacteria used an antibody–antibiotic conjugate consisting of a monoclonal antibody against teichoic acids bound to a rifamycin class antibiotic¹⁸⁸. This antibody–antibiotic conjugate binds to the cell surface and, upon opsonization, the proteolytic environment of the phagolysosome causes release of the active antibiotic form, allowing the targeting of intracellular organisms¹⁸⁸.

Local treatments have been tried with a varying degree of success in osteomyelitis therapy. The use of topical antibiotic therapy is controversial in the setting of osteomyelitis treatment. Traditionally, antibiotic-eluting beads have been used as a local antibiotic delivery source with variable effects on treatment outcomes depending on the anatomical location examined^{168–170}. The two-stage exchange for PJI, with a first-stage placement of an antibiotic eluting spacer, is considered the standard of care for chronic PJI; however, the necessity of the antibiotic elution from the spacer versus limb support and treatment of dead space (empty space from excised tissue) remains controversial. Similar to infection prevention, topical antibiotic powder may improve reinfection rates in combination with other local treatments such as dilute povidone-iodine after treatment of chronic PJI¹⁷¹. Other local treatments include the use of novel antimicrobial surface coatings. For example, silver-coated and gentamicin-coated prostheses have been used clinically in megaprotheses for large periarticular bone defects and for fracture fixation, respectively^{172,173}. Bacteriophage therapy has generated interest for many decades, and recent studies have investigated it as an adjuvant in the treatment of osteomyelitis¹⁷⁴. For example, in a murine model of implant-associated osteomyelitis, the use of a combination of systemic and locally applied bacteriophage-derived lysin (PlySs2), aimed at targeting both planktonic and biofilm-associated bacteria, improved *S. aureus* infection eradication after debridement and implant retention¹⁷⁵. Phage therapy has been used for topical delivery in humans for indications such as diabetic toe ulcers and limited cases of other lower extremity osteomyelitis; however, these studies involved small patient numbers without control groups¹³³.

Conclusions and perspectives

Based on the gravity of the clinical problem and advances in experimental, translational and clinical research technologies, there has been a resurgence of bone infection

research that has elucidated novel mechanisms of microbial pathogenesis, biofilm formation, protective versus pathogenic host immunity, and major challenges that need to be overcome for the development of effective diagnostics, vaccines and therapies. Moreover, agreement on these crucial issues has been established by international consensus^{176–179}. Although the bulk of this research is focused on *S. aureus*, based on its prevalence in clinical bone infections and the poor outcomes associated with these cases, studies of other challenging pathogens (for example, CoNS, *Streptococcus* spp., *Mycobacterium* spp. and *Pseudomonas* spp.) as well as of opportunistic bacteria in polymicrobial infections are active areas of investigation.

Currently, diagnosing bone infection remains a major challenge based on the insensitivity of standard-of-care cultures, which fail to detect indolent bacteria due to biopsy limitations, and ongoing antibiotic therapy that can cause false negative results. Although the advent of NGS has overcome some of these sensitivity issues, this diagnostic suffers from specificity issues related to contamination and our lack of understanding between alterations in the microbiome of polymicrobial infections and disease-causing microorganisms. Similarly, diagnostics of host factors have also improved but challenges of sensitivity and specificity remain. In the case of inflammatory cytokines (that is, c-reactive protein and α -defensin), detection of high levels of these proteins in serum and synovial fluid combined with clinical signs and symptoms have proven to be very effective diagnostics for infection but are incapable of determining the virulent organism. By contrast, assessment of pathogen-specific IgGs can provide specificity; however, immunological memory against the most common pathogens presents sensitivity challenges that may require longitudinal assessment of antigen-specific lymphocytes.

The recent elucidation of the distinct mechanisms of bacterial invasion, colonization and biofilm formation during the establishment of bone infection and chronic osteomyelitis highlight novel targets and approaches for prophylaxis and therapy. Based on the discovery of bacterial colonization of the OLCN, new approaches with bisphosphonate-conjugated antibiotics¹⁸⁰ and locally delivered microspheres¹⁸¹ to enhanced bone targeting and sustained antibiotics release are being tested. Additionally, there are several phage therapies being investigated as novel treatments for complicated bone infections¹⁸².

To overcome the dismal history of vaccine development for osteomyelitis, the field has taken a step back from approaches with accelerated development from antigen discovery to clinical trials, and has invested in sophisticated small, intermediate and clinically relevant animal models¹⁸³, which are designed with specific outcomes to quantify: in vivo bacterial growth with bioluminescence imaging¹⁸⁴ and fluorescent intravital microscopy¹⁸⁵, biofilm formation on bone and implants¹⁸⁶, osteolysis¹⁸⁷ and osseous integration⁶⁴, and human immune responses¹⁰⁵. From this work, the field has gained major insights into protective versus pathogenic immune responses during bone infection¹²¹, which has translational potential into prognostics¹¹⁸

and promising vaccines¹⁸⁸. With this knowledge, passive immunization with monoclonal antibodies to these bacterial targets in synergy with antibiotic treatment can now be investigated as therapies to reduce the very

high reinfection rates following revision surgery for implant-associated osteomyelitis.

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