**Bacterial Toxins in Musculoskeletal infections**

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**Abstract**

Musculoskeletal infections (MSKIs) remain a major health burden in orthopaedics. Bacterial toxins are foundational to pathogenesis in MSKI, but poorly understood by the community of providers that care for patients with MSKI, inducing an international group of microbiologists, infection disease internists, orthopaedic surgeons and biofilm scientists to review the literature in this field to identify key topics and compile the current knowledge on the role of toxins in MSKI, with the goal of illuminating potential impact on biofilm formation and dispersal as well as therapeutic strategies.

The group concluded that further research is needed to maximize our understanding of the effect of toxins on MSKIs, including:

1. further research to identify the roles of bacterial toxins in MSKIs,
2. establish the understanding of the importance of environmental and host factors and *in vivo* expression of toxins throughout the course of an infection,
3. establish the principles of drug-ability of anti-toxins as antimicrobial agents in MSKIs,
4. have well-defined metrics of success for anti-toxins as anti-infective drugs,
5. design a cocktail of anti-toxins against specific pathogens to (a) inhibit biofilm formation and (b) inhibit toxin release.

The applicability of anti-toxins as potential antimicrobials in the era of rising antibiotic resistance could meet the needs of day-to-day clinicians.

**Keywords**

Biofilm Meeting, Musculoskeletal Infection (MSKI), Bacterial Toxins, Virulence Factors, PJI, Staphylococcus

**Introduction**

Musculoskeletal infections (MSKIs) remain a major health burden in orthopaedics 1. Microbial virulence factors can play a major role in the pathogenies of MSKIs. Among these, toxins are imperative virulence factors. They can be defined as poisonous substances/ molecules that are produced by organisms that function autonomously and interfere with or directly harm the host. From the clinical perspective, bacterial toxins (exotoxins and endotoxins) are generally classified into three main categories:

1. Receptor-dependent or receptor-independent membrane-damaging toxins e.g. cytolytic toxins such as *Staphylococcus aureus* α-toxin and Panton-Valentine-Leukocidin (PVL)
2. Non-membrane-damaging toxins that interfere with receptor function e.g. toxic shock syndrome toxin (TSST) and a variety of enterotoxins
3. Secreted enzymes. These are enzymes that degrade host molecules or alter host metabolic pathways e.g. protease aureolysin

One strain of bacteria can produce a multitude of toxins simultaneously. Exotoxins are usually proteins, such as bacterial proteases and hyaluronidases that can degrade extracellular matrix or disrupt the proteins that hold epithelial cells together. Other toxins have systemic effects and many bacterial toxins contribute to the molecular machinery employed in biofilm formation and/or dispersal of microorganisms from mature biofilm. *Staphylococci* the most common causative species of MSKIs provide instructive examples of this toxin-biofilm interplay and hence a large section of this review is devoted to these organisms

Although, bacterial toxins are foundational to pathogenesis in MSKI, they are poorly understood by the community of providers that care for patients with MSKI, inducing an international group of microbiologists, infection disease, orthopaedic and biofilm experts to review the literature in this field and compile the current knowledge on the role of toxins in MSKI, with the goal of illuminating potential impact on biofilm formation, dispersal as well as therapeutic strategies for MSKIs.

**Methodology**

A biofilm symposium was held at the Hospital for Special Surgery in New York, NY, USA on August 1st 2019, and the contents of the discussion on bacterial toxins in MSKI was discussed with the Editor-in-Chief of the *Journal of Orthopaedic Research*. Following a presentation on the topic (by KS) in the symposium, experts who attended the meeting were invited to participate and come up with key topics related to this area and contribute to a review manuscript. The willing participants (co-authors) identified topics related areas of interest and unknowns in the field of toxins and MSKIs. Contributors provided draft topics to the lead author (KS), a manuscript was drafted and sent back to all authors for further comments, suggestions and agreement was obtained on the final version of the article before submission.

This work focuses mainly on toxins that play important roles in pathogenesis and present treatment opportunities. Additionally as *Staphylococci,* the most common causative species of MSKIs, provide instructive examples of toxin-biofilm interplay and a large section of this review is devoted to these organisms.

**Results:**

The following key topics were selected and reviewed:

1. ***Staphylococcal* species**

**1.A Staphylococcal Toxins and MSKIs**

*Staphylococcus aureus* [methicillin-sensitive (MSSA); methicillin-resistant(MRSA) *S. aureus*] and coagulase-negative staphylococci (CoNS) belong to the most common causes of MSKIs. Although *S. aureus* infection can be associated with florid inflammation and CoNS with a low-grade presentation, the organisms can reveal a converse clinical pattern. Moreover, *S. aureus* can still be isolated in reactivated osteomyelitis after decades of remission 2.

There are a multitude of *S. aureus* virulence factors with activities such as degradation of antimicrobial peptides and inhibition of complement mediated destruction. Virulence factors include the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that allow interactions with proteins such as collagen, fibronectin, and fibrinogen, and are integral to biofilm formation along with staphylococcal autolysins. These proteins perform critical roles in their interaction with the extracellular matrix (ECM) and serum proteins by either coating implants or contributing to aggregation in the synovial fluid 3 to result in prosthesis- or tissue-adherent biofilms or floating biofilms formed on a fibrous proteinaceous matrix 4.

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, in MSKI, are to break down host tissue and provide nutrients for bacterial survival and growth (table 1) 5-16 .

**1.B Factors associated with the aggregation of *S. aureus* in synovial fluid.**

Biofilm-associated MSKI is a clinically challenging 17, the biofilm that is formed in synovial fluid (rich in serum and ECM proteins) is unlike the biofilm observed *in vitro*. It is defined by the binding of ECM/serum proteins by bacteria to form fibrinogen-containing biofilms/aggregates 18. Aggregated bacterial biofilm that forms with *S. aureus* and other bacterial strains in multiple types of synovial fluid (bovine, porcine, equine, human; normal, diseased). As fibrin[ogen] is abundant in diseased joints 19, it appears to be the key component of the aggregate. Pre-digestion of the synovial fluid with plasmin blocks *S. aureus*  aggregation, 18 and treatment of existing aggregates by tissue plasminogen activator (TPA) seems to disperse aggregates 20, suggesting that interactions with serum proteins such as fibrinogen are important for aggregation. In fact, when the bacterial proteins responsible for binding to the serum proteins fibrinogen, albumin, and fibronectin (fibronectin binding protein A and B (FnbA and FnbB)  and clumping factor A and B (ClfA and ClfB)) are inactivated due to gene insertions, the ability of MRSA to form aggregates in synovial fluid is attenuated or eliminated 21.

When Staphylococci reach a critical mass at an infection site, cell-to-cell communication via peptide signaling can activate a series of cellular processes by a mechanism known as “quorum sensing”. Mutations in the MSCRAMMs can moderate the ability of *S. aureus* to cause infection 22. The accessory gene regulator (*agr*) system controls MSCRAMM expression and regulates the quorum sensing system along with the P2 and P3 promoters. In turn, they regulate a variety of cell surface and secreted proteins, notably the PSMs that are critical to the aggregatory phenotype21. In synovial fluid, *agr* and its downstream effectors the P2 and P3 promoters, as well as PSMα and β show reduced expression 21, showing that interactions with the synovial fluid actively modify the phenotype of the bacteria. Even in synovial fluid, aggregation can be reduced by over expression of the surfactant-like PSMα or PSMβ in MRSA USA300, suggesting that this *agr*-mediated suppression is critical in aggregation. Interestingly, preclinical studies have confirmed that *agr* is required for *S. aureus* emigration from implant-associated biofilm, but is not required for colonization of osteocytic-canalicular networks during chronic osteomyelitis 23.

Because aggregation of *S. aureus* within synovial fluid alters both quorum sensing and virulence factor/toxin production, it seems likely that the MRSA/MSSA aggregate represents a less virulent infection. With bacterial release from the aggregate, the polysaccharide intracellular adhesin (PIA) coating of the aggregate is released, and there should be a marked increase in virulence factor/ toxin expression—with an accompanying increase in antibiotic sensitivity. Thus, disaggregation resulting in increased *agr*-mediated gene expression of the deleterious virulence factors/toxins restores antibiotic susceptibility and by implication, the possibility of bacterial eradication.

**1.C Biofilm and exotoxin interactions**

*S. aureus* has an inverse relationship between biofilm formation and exotoxins production/ secretion and other virulence factors. There is evidence for both direct and inverse relationships that potentially allows *S. aureus* to fine-tune its survival in the host. *S. aureus* biofilm can persist in multiple phenotypes by which it can evade detection by the host and clinical microbiology or attack the host during episodes of proliferation.

These phenotypes have been associated with phases of infection severity. Five distinct stages in the development of biofilm formation by *S. aureus* have been identified (figure 1) 24:

1. attachment of cells to a surface
2. multiplication of these cells into a thin layer and production of an extracellular polymeric substance (EPS), or slime, matrix consisting of proteins and extracellular DNA (eDNA) which holds the cells together and anchors the developing biofilm to the surface
3. exodus in which some cells are released through degradation of the eDNA by enzymatic extracellular nuclease activity
4. maturation into larger microcolonies which triggers the density dependent *agr* quorum sensing system to begin to produce proteases which break down the proteinaceous component of the EPS and PSMs (which have a surfactant effect) that leads to the final phase
5. dispersal in which cells and aggregates are released from the biofilm into the surrounding fluid.

Suppression of PSMs has been associated with the ability of *S. aureus* to form large suspended aggregates in synovial fluid, with an antibiotic or antimicrobial tolerant biofilm-like phenotype 21.

The staphylococcal accessory regulator (SarA) protein, has similarly been shown to regulate the production of secreted virulence factors such as α-toxin while repressing biofilm formation 25. Interestingly the *saePQRS* regulatory system, a third system that regulates biofilm formation and toxin production (including α-toxin) has been shown to promote biofilm formation by limiting the production of extracellular proteases that degrade the biofilm EPS 26.

Although the above mentioned 5-stage model suggests a linear timeline of development, it is not clear how long each stage may persist in Periprosthetic joint infections (PJI). It is possible that the biofilm can cycle through the more symptomatically quiescent stages of multiplication to the more aggressive “flare-ups” caused by the dispersal stage. Such cycling could explain anecdotal evidence that suggests that the biofilm can reside in the quiescent stages for months or even years before manifesting as an initial or recurrent severe localized or systemic infection 2.

*S. aureus* α-toxin (table 1) contributes to the formation of biofilms on mucosal surfaces by disrupting the host epithelium 27.β-toxin of *S. aureus* is a neutral sphingomyelinase (SMase). β-toxin plays a key role in the establishment of staphylococcal biofilms. However, *S. aureus* biofilms can be made by many strains that do not produce β-toxin, requiring other factors dependent upon the specific strain. These factors include cell surface virulence factors, extracellular polysaccharides, other proteins, and extracellular DNA, all pointing to a molecular mechanism for forming the structural framework of many staphylococcal biofilms.

**1.D Influence of the environment and surfaces on the production of staphylococcal exotoxins**

The detection of multiple toxins (HlgB, LukD/E and α-toxin) in biofilms of multiple strains on Leiden human epidermal models (LEMs), but not on polystyrene surfaces (PS), indicates surface specific protein expression. This suggests that currently used biofilm models, might not adequately reflect biofilm formation on more complex surfaces, such as the human skin. The detection of *S. aureus* toxins, most notably α-toxin, in biofilms on LEMs is in agreement with their well-established roles in the pathogenesis of skin infections. The cytolytic pore-forming α-toxin lyses human cells, interferes with the innate and adaptive immune responses in a murine skin infection model, and is essential for biofilm development on mucosal surfaces. In human skin, the filaggrin protein may inhibit α-toxin’s cytotoxicity by its ability to regulate the secretion of SMase. Other toxins detected including gamma-haemolysin component B precursor (HlgB) and the leukocidins D and E (LukD and LukE), have also been associated with *S. aureus* skin colonization and infection. Regulation of *Hla* and other genes for *S. aureus* virulence factors is influenced by many factors, including the agr, RNAIII, downstream transcription factors Rot, SarA and SarS and Sae. Therefore, it may well be that the quorum sensing system of AgrA/RNAIII of *S. aureus* is activated differently upon interaction of *S. aureus* with either LEMs or PS, leading to up- or down-regulation of specific genes depending on the surface 28.

*S. aureus* has intricate layers of regulation that control the release of virulence factors. The *agr* regulatory system controls a myriad of secreted toxins, and identifying the exact agents responsible for dispersal has represented a challenge. Early studies suggested that surfactant properties of delta-toxin could be responsible considering that many bacterially produced surfactants have known anti-biofilm effects, as it became evident that delta-toxin was part of a larger family of PSM peptides. Numerous environmental and metabolic factors such as pH, reactive oxygen species, and nutrient availability, can modulate *agr* quorum sensing in *S. aureus.* The mechanisms by which these factors impact quorum sensing are diverse, but can be organized roughly into three broad categories: (i) suppression of quorum sensing through the action of reactive oxygen species, generated by innate immune cells on components of the *agr* system; (ii) transcriptional up- or down- regulation of *agr* expression as a consequence of various non-*agr* regulatory proteins binding within the *agr* P2-P3 promoter region; and (iii) the up- or down-regulation of *agr* expression by environmental factors for which the mechanism of action is yet to be identified 29.

**1.E Understanding Bacterial Toxins, Humoral Immune Responses in MSKIs: potential diagnostic and therapeutic strategies?**

*S. aureus*‘ repertoire of toxins and proteases targets elements of the innate and adaptive immune responses. For example, a recent report described the priming of regulatory T-cells initiated by the action of PSMs on human dendritic cells 30; this is of importance in MSKIs. PSMs have been shown in murine and human models to modulate human monocyte-derived dendritic cells to prime regulatory T cells, thus muting the local adaptive immune response and increasing tolerance to the bacteria 30. One possible mechanism for this phenomenon is the ability of PSMs to block the p38-CREB pathway in dendritic cells, impairing cytokine production, and therefore leading to regulatory T cell priming 31.

Humans express IgG antibodies against numerous *S. aureus* antigens in their serum. During acute MSKIs, antibody levels for specific antigens increase 2 to 10 fold 32. Most of the antibodies are specific for the secreted products/ enzymes displayed on the cell wall 33. Further, these antibodies are maintained at relatively high levels even in non-infected patients, but it is not clear that they provide protection against new infections. How and if patients clear *S. aureus* infections, and the role played by circulating antibodies, remain topics of considerable debate, but there are three practical roles for antibodies in patient care:

1. **Utility of antibodies as diagnostic biomarkers**. Antibody levels do increase in new infections, and that increase can be utilized as a measure of ongoing infection. In MSKIs, serum antibody levels for particular antigens, especially Iron-regulated surface determinant system-B (IsdB), rise substantially providing a possible diagnostic tool 34. However, the utility of this approach is limited by: a) the huge range of antibody levels in non-infected patients; and b) the fact that, once elevated, anti-*S. aureus* antibody levels decline slowly over months. Similar diagnostic power can be obtained using the recently stimulated circulating plasma cell population, antibody-secreting cells (ASC), which can be cultured *in vitro* to express their antibodies into a cell culture fluid termed medium enriched for newly synthesized antibodies (MENSA) 35–37. For diagnostic purposes, MENSA has a major advantage, because there is no pre-existing antibody from prior exposures, so the analytic background is practically zero.
2. **Utility of antibodies as prognostic tools.** The presence of ASC in the circulation is a measure of the ongoing immune response. ASC actually emerge earlier than the rise of antibody levels in the serum. They decline once the pathogen is defeated making them potential tools for monitoring response to therapy.
3. **Utility of antibodies as therapeutic agents**. It is uncertain if and why the abundant anti-*S. aureus* antibodies in most patients’ sera provide so little protection against infection. Numerous investigators have brought forward new candidate antibodies for passive immunological protection and antigens for active vaccines. Each has demonstrated potential in animal models, but none has exhibited utility in human trials. However, antibody-based therapeutics continue to be pursued. The identification of ADAM10 as a cellular receptor for α-toxin has provided keen insight on the biology of the toxin’s action during disease pathogenesis, demonstrating the molecular mechanisms by which the toxin causes tissue barrier disruption of host interfaces lined by epithelial or endothelial cells. The use of anti-α-toxin has been shown to induce passive immunity against pneumonia in a mouse model 38. In addition, the use of small molecular ADAM10 inhibitors have shown promise in experimental models in sepsis 39.

In MSKIs, the potential for biofilm disruption with antitoxin is being studied 40. Testing of the 4-C *Staph* vaccine demonstrated strong antibody response and reduction in *S. aureus* bacterial load in a murine model of septic arthritis 41. It is worth mentioning that the clinical trial for the staphylococcal vaccine from *Pfizer* had to be stopped, because of the low statistical probability for the study to meet primary efficacy objectives and not safety issues according to the conclusion of the Data Monitoring Committee.

Furthermore, animal studies has suggested that the *agr* system is important in MSKIs 42. Conversely, Vuong *et.al,.* demonstrated that an *agr*-deficient strain of *S. epidermidis* showed enhanced biofilm development in an infected implant rabbit model 43. They also studied 53 *S. epidermidis* strains isolated from patients with PJIs and 21 strains isolated from the skin of healthy individuals. Strains that showed a non-functioning quorum sensing system, suggesting a non-functioning *agr* system, were found in 36% of PJIs and only in ~ 5% of the healthy samples. Valour *et.al.,* performed a similar evaluation of *S. aureus* isolates obtained from acute and chronic cases of human MSKIs. Isolates were screend for delta-toxin production, which is a product of the RNA III transcript that is up-regulated by the *agr* system, and the authors found a lack of delta-toxin production in ~26% of chronic infections compared with ~11% of acute infections. No difference was noted in the virulence gene distribution or biofilm formation between the acute and chronic groups 44. Such data potentially highlight the difference between *in-vitro* and *in-vivo* studies. Alternatively, it may simply highlight the changing necessity of various factors/systems in the establishment of biofilms. The latter idea has particular therapeutic implications e.g., treatment with RNA III-inhibiting-peptide may inhibit/prevent biofilm formation in the early stages of staphylococcal infection, but not in chronic infections.

There are also monoclonal antibodies that, by neutralizing specific targets such as α-hemolysin 45 or the cell wall enzyme glucosaminidase 46, may augment host defences. Combinations of antigens or monoclonal antibodies have been sought in frank recognition of the ability of *S. aureus* to shift its antigenic repertoire 47. Finally, there is the hope of introducing a new class of recognition molecules called centyrins that can be selected from variants built on an immunoglobulin-like domain of the human protein tenascin-C 48.

**2. *Streptococcus* species**

Beta-haemolytic streptococci are the most commonly described streptococcal species in MSKIs. While group A *Streptococcus* (GAS) or *S.* *pyogenes* are prevalent in children, group B *Streptococcus* (GBS, *S. agalactiae*) play an increasing role in elderly/ adults with comorbidities. In the past decades, a growing incidence of MSKIs due to group C and G *Streptococcus* (GCGS, S*. dysgalactiae* sup. *equisimilis*), in the elderly, has been noted. However, molecular investigations have not yet identified a specific factor that could explain the arthritogenicity of GCGS.

GAS and GCGS have several virulence determinants in common, including Streptolysin O and S, Streptococcal pyrogenic exotoxins [Spe] and M protein. M proteins and pili-like structures play a role in adherence to host cells and biofilm formation. M protein, SpeA and SpeB induce human mononuclear cells to produce TNF-α, IL-1 and IL-6. Thereby, they trigger a severe inflammatory response, eventually leading to the destruction of chondrocytes and osteoblasts. In a mouse model, GAS infection of osteoblasts induces receptor activator of NF-kappa-B-ligand (RANKL) expression 49 that contributes to bone destruction. GBS consists of several virulence factors that are involved in the pathogenesis of MSKIs; β-hemolysin/cytolysin (β-h/c) toxin is associated with pigment expression. It is hemolytic and cytolytic due to its pore-forming capacities 50–52 and triggers cytokine release. Hyperhemolytic GBS strains are considered to be frequently, but not uniformly more pathogenic compared to nonhemolytic strains 53–55.

**3. *Cutibacterium acnes***

*Cutibacterium acnes* (*C. acnes*) [formerly *Propionibacterium acnes*] is often an indolent cause of MSKIs. There is a possibility that pore-forming toxin, like the α-hemolysin of *S. aureus*, contributes to the pathogenesis and/ or symptomatology of chronic *C. acnes* disc infections. Hyperhemolysis on blood agar has been proposed as a microbiological marker for MSKIs 56–59.

**4. *Clostridium perfringens***

Gas gangrene (Clostridial myonecrosis) is a life-threatening infection, typically, but not exclusively caused by *Clostridium perfringens*. Among the toxins that are produced by *C. perfringens*, the alpha and theta-toxins are known to be major virulence factors. The α-toxin is haemolytic and has phospholipase C and SMase activities. It inhibits the differentiation of neutrophilic granulocyte cells and impairs erythropoiesis. The theta-toxin, also known as perfringolysin O, is a member of the cholesterol-dependent cytolysin family and is pore-forming. MSKIs caused by *Clostridium* spp. are rare, but include septic arthritis, PJI, postoperative wound infections and spondylodiscitis.

In comparison to gas gangrene that is characterized by the destruction of muscle accompanied with organ failure and shock, clostridial arthritis presents with classic features of pyogenic joint infection. Radiological signs of intra-articular gas are infrequently reported. In fracture-related infections caused by *Clostridium* spp., there is often involvement of multiple organisms (i.e.; polymicrobial infections) and these patients have a higher likelihood of complications, including delayed wound healing or development of a septic non-union.

Despite the difference in clinical presentation between gas gangrene and osteoarticular infection, there are pathogenic similarities in these clostridial infections, mainly triggered by their toxins. In an experimental model, intramuscular injection of a clostridial toxin preparation containing both phospholipase C and theta-toxin decreased the blood flow rapidly and irreversibly via formation of intravascular aggregates that obstructed the blood vessels. The toxins worked synergistically for bacterial survival and the spread of disease in gas gangrene 60. The perfusion deficits led to an anaerobic environment, which was an ideal niche for bacterial persistence. This process possibly prevents bone healing and contributes to tissue necrosis, and eventually to the development of a non-union in fractured bones. However, the effect of *C. perfingens* toxins on osteoblasts and chondrocytes have not yet been reported. The absence of tissue inflammatory response is another characteristic observed in clostridial infections 61. In addition to the hindered blood flow which impairs the movement of inflammatory cells to the infected tissue, alpha and theta-toxin also destroy leukocytes due to their cytotoxic properties 62.

**5. *Kingella kingae***

Kingella kingae is an increasingly identified cause of MSKIs typically in children less than 4 years of age. The true incidence of paediatric K. kingae septic arthritis is unknown, as the clinical presentation may mimic transient synovitis or rheumatologic conditions, and the organism is often not detected by routine culture techniques. While pili play a role in adherence to host cells, K. kingae ‘s RTX toxin may help the invasion to skeletal tissues and has a wide-spectrum of cytotoxic effects, including to synovial cells 63.  Additionally, marked reduction in the circulating white blood cells could be a strategy to guarantee *K. kingae*'s subsistence in the host's bloodstream, skeletal tissues, and endocardium. Disruption of the K. kingae RTX locus resulted in a loss of cytotoxicity for respiratory epithelial, synovial, and macrophage cell lines, and the absence of RTX toxin in species like *K. denitrificans* and *K. oralis* rendered them less virulent 64.

**6. *Pasteurella multocida***

*Pasteurella multocida*toxin (PMT) is a potent toxin involved in bone destruction. Experiments in animal models demonstrated that PMT manipulate host cell signaling cascades resulting in the activation of mitogenic pathways and the production of cytokines for many cell types; leading to excessive bone degradation.

PMT-mediated bone damage is independent of RANKL, a cytokine thought to be crucial for triggering osteoclastogenesis. When RANKL plus osteoprotegerin are added to PMT-treated macrophages, no effect on PMT-induced osteoclast formation is observed 65. PMT mediates the release of osteoclastogenic cytokines such as IL-6 and TNF-α, but not IL-1, which supports the differentiation process. An *in-vitro* study using etanercept (anti-TNF) strongly reduced PMT-induced osteoclast formation. Similarly blocking IL-6 also led to a reduction in PMT-induced osteoclast formation in macrophages 66–68, these may be used as potential therapeutic strategies in the management of *P. multocida*osteomyelitis.

**Conclusion and future directions**

We have presented here a number of topics in a thought-provoking manner related to bacterial toxins and their roles in MSKIs. However, the role of toxins and other virulence factors among other bacteria that can cause MSKIs (e.g. Salmonella spp., Mycobacteria spp., *Neisseria gonorrhoea*, *Borrelia burgdorferi*) have not been discussed in this review. There are many unknowns on the effect of toxins on osteocytes, chondrocytes, biofilm formation and MSKIs and persistent infections. Identification of the interaction between toxins and antitoxins at the molecular level coupled with the interaction of human immune responses will be critical to the understanding of the impact in pathogenesis and the production of next-generation antimicrobial molecules, anti-toxins and vaccines to manage MSKIs.

Further studies, to maximize our understanding of the effect of toxins on MSKIs, are needed including:

1. research to identify the roles of bacterial toxins in MSKIs,
2. establish the understanding of environmental and host factors and *in vivo* expression of toxins throughout the course of an infection,
3. establish the principles of drug-ability of antitoxins as antimicrobial agents in MSKIs,
4. have well-defined metrics of success for anti-toxins as anti-infective drugs,
5. design cocktail(s) of anti-toxins against specific pathogens to (a) inhibit biofilm formation and (b) inhibit toxin release. This may also reduce or eliminate impact on selection pressure or the microbiome compared to traditional antibiotics.

The applicability of anti-toxins as potential antimicrobials in the era of rising antibiotic resistance could meet the needs of day-to-day clinicians.

**Glossary**

ADAM10 A disintegrin and metalloproteinase

*agr* Accessory gene regulator

ASC Antibody-secreting cells

Clf Clumping factor

ECM Extracellular matrix

eDNA Extracellular DNA

EPS Extracellular polymeric substance

FnbA or B Fibronectin binding protein A or B

GAS Group A *Streptococcus*

GBS Group B *Streptococcus*

GCGS Group C and G *Streptococcus*

GGS Group G *Streptococcus*

Hla Alpha-hemolysin

IsdB Iron-regulated surface determinant system-B

LEMs Leiden human epidermal models

Luk Leukocidins

MENSA Medium enriched for newly synthesized antibodies

MSCRAMMs Microbial surface components recognizing adhesive matrix molecules

P2 or P3 Promotors

p38-CREB p38-cAMP response element binding

PIA Polysaccharide intracellular adhesion

PMT *Pasteurella multocida* toxin

PSMs Phenol soluble modulins

RANKL Receptor activator of NF-kappaB ligand

RTX  Repeat-in-toxin

SaePQRS *S. aureus* exoprotein regulatory system

SarA Staphylococcal accessory regulator

SMase Sphingomyelinase

Spe Streptococcal pyrogenic exotoxins

TPA Tissue plasminogen activator

TSST-1 Toxic shock syndrome toxin 1

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