Staphylococcus aureus Toxins and Diabetic Foot Ulcers: Role in Pathogenesis and Interest in Diagnosis

Catherine Dunyach-Remy [1,2], Christelle Ngba Essebe [1], Albert Sotto [1,3] and Jean-Philippe Lavigne [1,2,*]

[1] Institut National de la Santé Et de la Recherche Médicale U1047, Université de Montpellier, UFR de Médecine, Nîmes 30908, France; catherine.remy@chu-nimes.fr (C.D.-R.); ngbachristelle@hotmail.fr (C.N.E.); albert.sotto@chu-nimes.fr (A.S.)

[2] Service de Microbiologie, Centre Hospitalo-Universitaire Carémeau, Nîmes 30029, France

[3] Service des Maladies Infectieuses et Tropicales, Centre Hospitalo-Universitaire Carémeau, Nîmes 30029, France

* Correspondence: jean.philippe.lavigne@chu-nimes.fr; Tel.: +33-466-683-202

Abstract: Infection of foot ulcers is a common, often severe and costly complication in diabetes. Diabetic foot infections (DFI) are mainly polymicrobial, and *Staphylococcus aureus* is the most frequent pathogen isolated. The numerous virulence factors and toxins produced by *S. aureus* during an infection are well characterized. However, some particular features could be observed in DFI. The aim of this review is to describe the role of *S. aureus* in DFI and the implication of its toxins in the establishment of the infection. Studies on this issue have helped to distinguish two *S. aureus* populations in DFI: toxinogenic *S. aureus* strains (harboring exfoliatin-, EDIN-, PVL- or TSST-encoding genes) and non-toxinogenic strains. Toxinogenic strains are often present in infections with a more severe grade and systemic impact, whereas non-toxinogenic strains seem to remain localized in deep structures and bone involving diabetic foot osteomyelitis. Testing the virulence profile of bacteria seems to be a promising way to predict the behavior of *S. aureus* in the chronic wounds.

Keywords: diabetic foot infection; *Staphylococcus aureus*; toxins

1. Introduction

Foot ulcers are common in diabetic patients. Its prevalence varies between 15% and 25% [1]. Infection of these ulcers is a frequent (40%–80%) complication representing a major cause of mortality and morbidity [2]. It is estimated to be the most common reason of lower-limb amputations [3–5]. The pathophysiology of diabetic foot infection (DFI) is quite complex. The prevalence and severity are a consequence of host-related processes (e.g., immunopathy, neuropathy and arteriopathy) and pathogen-related factors (e.g., virulence, antibiotic-resistance and microbial organization) (Figure 1) [6–8].

DFI pose many problems in clinical practice in terms of both management and diagnosis [9]. Indeed, the presence of impaired leukocyte functions and/or peripheral arterial disease may reduce the local inflammatory response and classical signs or symptoms of local infection [10,11]. Moreover, systemic signs of toxicity (e.g., leukocytosis or fever) may be lacking or appear late, even in severe cases [12–14]. Microbiological diagnosis of these DFI also encounters many limitations. As microorganisms colonize all chronic wounds, the diagnosis of DFI should not be based only on the microbiological analysis of a wound culture, but also on clinical findings [5,9,15]. The Infectious Diseases Society of America (IDSA) and the International Working Group on the Diabetic Foot (IWGDF) have developed clinical criteria for classifying the severity of DFI (Table 1) [15,16].
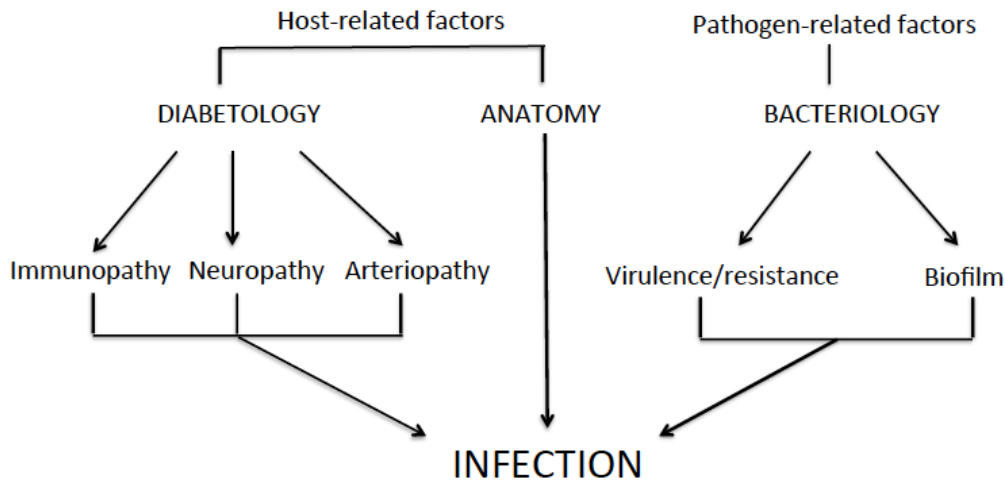
Figure 1. Interactions between metabolic, anatomical and bacteriological factors in diabetic foot infection.

Table 1. International Consensus on the Diabetic Foot classification of foot wound infections [3,15].

Grades	Symptoms
Grade 1	No symptoms, no signs of infection
Grade 2	Lesion only involving the skin (no subcutaneous tissue lesion or systemic disorders) with at least two of the following signs: - local warmth - erythema >0.5–2 cm around the ulcer - local tenderness or pain - local swelling or induration - purulent discharge (thick, opaque to white or sanguineous secretion) Other causes of inflammation of the skin must be eliminated (for example: trauma, gout, acute Charcot foot, fracture, thrombosis, venous stasis)
Grade 3	- Erythema >2 cm and one of the findings described above or - Infection involving structures beneath the skin and subcutaneous tissue, such as deep abscess, lymphangitis, osteomyelitis, septic arthritis or fasciitis There must not be any systemic inflammatory response (see Grade 4)
Grade 4	Regardless of the local infection, in the presence of systemic signs corresponding to at least two of the following characteristics: - temperature >39 °C or <36 °C - pulse >90 bpm - respiratory rate >0/min - $PaCO_2$ <32 mmHg - leukocytes >12,000 or <4000/mm^3 - 10% of immature leukocytes

For many decades, culturing wound specimens were the only way to determine the causative pathogen(s) in a DFI. As microorganisms are always present on every skin wound and the DFI are often polymicrobial, the variability of bacterial virulence factors and the level of host resistance must also be taken into account. In fact, the different organisms isolated from infected wounds do not have a similar pathogenic impact, and evaluation of the intrinsic virulence potential of isolated bacteria to identify their real pathogenicity seems a promising way to best characterize the infection and to distinguish infection from colonization [16].

Several studies have shown that DFI are polymicrobial, and *Staphylococcus aureus* is the pathogen most frequently isolated [17–22]. *S. aureus* is both a commensal bacterium and a human pathogen.

Indeed, approximately 30% of the human population is colonized with *S. aureus* [23]. Importantly, this bacterium causes a wide range of clinical infections (e.g., bacteremia, endocarditis, skin and soft tissue, osteoarticular, pulmonary and device-related infections) [24]. The numerous virulence factors and toxins produced by *S. aureus* during infection are well characterized [22]. However, some specific features could be observed in DFI. The aim of this review is to describe the role of *S. aureus* in DFI and the implication of its toxins in the establishment of the infection.

2. DFI and *Staphylococcus aureus*

2.1. Clinical Aspects of DFI

Many DFIs are superficial at presentation. However, bacteria can spread to subcutaneous tissues, including tendons, joints, fascia, muscle and bone. DFIs were classified by their clinical severity, ranging from mild (~35% of cases, depending on site of presentation), through moderate (~30%–60%), to severe (~5%–25%) [25]. The IWGDF and the IDSA have proposed simple clinical criteria for classifying the infection of diabetic foot ulcer (DFU) based on classical signs and symptoms of inflammation (Table 1) [3,15,25–27]. This scheme helps to predict whether hospitalization would be required and the clinical outcome. Moreover, various factors have been suggested as markers of DFI when classical signs are not obvious. These include the identification of friable or discolored granulation tissue, necrosis, fetid odor, non-purulent secretions, delay in healing despite otherwise adequate ulcer management and the discovery of unexplained hyperglycemia [16,25]. Interestingly, neither toxic shock syndrome nor toxinogenic manifestations could be clearly diagnosed in DFI.

2.2. Osteomyelitis

Infection of bone, or osteomyelitis, is found in ~50%–60% of patients hospitalized for a DFI and ~10%–20% of apparently less severe infections presenting in the ambulatory setting [28]. Bone infection results in inflammatory destruction, bone necrosis and new bone formation. It typically involves the forefoot and develops by contiguous spread from overlying soft tissue, penetration through the cortical bone and into the medullary cavity. The clinical presentation of diabetic foot osteomyelitis (DFOM) can vary with the site, the presence of any associated abscess or soft tissue involvement, the extent of bone infection, the bacterial species and the adequacy of limb perfusion. Osteomyelitis is frequently associated with vascular insufficiency [24,28,29].

Two clinical manifestations are frequently found during DFOM: an ulcer lying over a bony prominence (particularly when it fails to heal despite adequate off-loading) and a 'sausage toe'. Osteomyelitis can, however, occur in the absence of overlying local signs of inflammation [30].

2.3. Matrix Metalloproteinases and DFI

During DFI, wound healing is hampered by mechanisms including a low growth factor activity, a reduced cellular proliferation, an elevated inflammatory markers and high levels of proteases [31].

The proteases are enzymes that act to control the degradation of extracellular matrix (ECM) [32]. The major group of proteases involved in the wound healing process are the matrix metalloproteinases (MMP) (e.g., MMP-2, MMP-8, MMP-9 and the serine proteases (human neutrophil elastase, HNE)). MMPs are endopeptidases whose physiological functions are to degrade the different components of the cutaneous tissue (collagen type I, elastin, etc.) and to facilitate keratinocyte migration and re-epithelialization [33,34]. HNE is an enzyme acting on a wide range of proteins in the ECM and on inflammatory mediators [31]. The MMPs' activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs) [35]. The balance between the level of proteases and their inhibitors is essential to allow a physiological healing process [36].

In DFI, under the hypoxic and inflammatory environment, the presence of elevated levels of MMPs could be noted as opposed to decreased levels of TIMPs [36–39]. This elevated protease activity participates in the important destruction of the ulcer ECM. It impairs the release of the different

factors regulating the wound healing process (the collagen synthesis is deregulated; the growth factor synthesis and action are stopped) and affects extracellular matrix components, such as fibronectin [40]. All of these elements stall the wound in a chronic inflammatory phase without progressing to healing. However, no clear link between delayed healing and elevated protease activity has been described. This reinforces the need to understand the organization/cooperation between bacteria species that can modulate local inflammation and host MPP production.

In addition to human MMPs, some bacteria produce proteases that have a role in the healing of infected wounds. For example, the zinc-metalloproteinase, elastase, produced by *Pseudomonas aeruginosa*, induces degradation of fibroblast proteins and proteoglycans in chronic wounds and has also been shown to degrade host immune cell mediators. The microbial proteases participate also in the degradation of human ulcer fluid and inhibit fibroblast growth. It has now been suggested that human and bacterial MMPs act synergistically to maintain the lesion in a chronicity status [32]. Many pathogenic bacteria isolated on wounds (including *S. aureus*) are able to produce metalloproteinases.

2.4. Prevalence of S. aureus in DFIs

In Occidental countries, Gram-positive aerobic cocci are the main microorganisms responsible for DFI with *S. aureus* the most commonly isolated bacteria, alone or in combination, in superficial or deep infection (Figure 2) [19,20,29,30,41–72]. In warmer countries (particularly in Asia and Africa), Gram-negative bacilli are more prevalent.
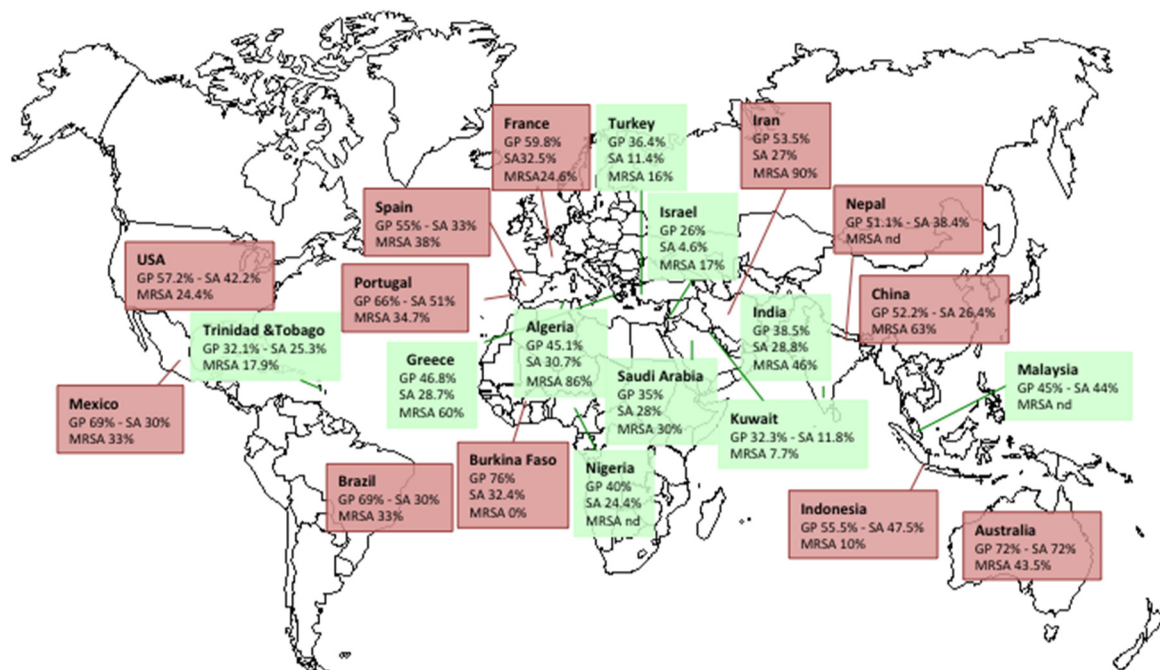
Figure 2. Worldwide geographic distribution of Gram-positive cocci (GP), *S. aureus* and Methicillin Resistant *S. aureus* (MRSA) isolated from diabetic foot ulcers. Red shading indicates regions where GP are predominant. Green shading indicates regions where Gram-negative bacilli are predominant.

Many cases of deep infections and DFOM are polymicrobial. Also in this case, *S. aureus* is the main isolated bacteria, present in 30%–60% of cases [25].

2.5. Resistance of S. aureus in DFIs

The prevalence of methicillin-resistant *S. aureus* (MRSA) in DFI varies among countries with an exacerbation in countries that are less developed (Figure 2) [19,20,28–30,41–73]. MRSA are more often

isolated from patients who have been previously hospitalized or reside in a chronic care facility, who have recently received antibiotic therapy or who have had a previous amputation [41,74].

In France, the prevalence of MRSA increased since the late 1990s [73,75]. Around 2005, different protocols were developed. National guidelines were implemented for the better management of DFI, notably concerning the debridement procedures, the microbiological samplings and antibiotic use [76]. The results of these guidelines entailed a significant decrease in the number of bacteria isolated per sample, in the increased rate of Gram-positive cocci and in the prevalence rate of multidrug-resistant bacteria, notably MRSA [3,15,28,77]. Concomitantly, hospital infection control measures have been improved [42,78,79], notably on the use of hydro-alcoholic solution for handwashing [80]. These measures have been associated with a reduction in MRSA diffusion [41].

For some authors, the isolation of MRSA in DFIs would be associated with more severe infections. However, different articles showed a similar clinical presentation and outcomes between MRSA and other pathogens [81–83].

Finally, some cases of DFIs due to vancomycin-resistant *S. aureus* have been described [84,85]. This type of resistance remains uncommon.

2.6. Pathogenesis

The pathogenesis of *S. aureus* in DFI is classical and corresponds to the physiopathology of skin and soft tissue infection (SSTI) [24,86]. The first defense against *S. aureus* infection is the neutrophil response. When *S. aureus* enters the injured skin, neutrophils and macrophages migrate to the site of infection. *S. aureus* evades this response using different methods (e.g., blocking sequestering host antibodies, chemotaxis of leukocytes, hiding from detection via capsule or biofilm formation and resisting destruction after ingestion by phagocytes).

The knowledge of *S. aureus* pathogenicity reveals that these bacteria seem to be adapted for soft tissue and bone infections. Indeed the majority of infections remain localized to the feet. Generally, systemic infection secondary to diabetic foot is less prevalent (around 10%). This becomes particularly noticeable when analyzing the infection process [87]. The first event at the beginning of DFI is the adhesion to surface components (fibrinogen, fibronectin and epidermal keratinocytes). *S. aureus* attachment to ulcer surface depends on bacterial expression of numerous surface proteins that mediate adherence to components of bone matrix and collagen [88]. These bacterial cell surface receptors correspond to adhesins or microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [89–92] (Figure 3). MSCRAMMs facilitate bacterial adhesion to skin tissue. Moreover adhesins are essential in intracellular bone invasion. Indeed, *S. aureus* can invade osteoblasts [93], fibroblasts and endothelial cells. In the intracellular compartment this bacterium forms small-colony variants (SCVs) [94]. Thus, they are able to survive in a metabolically inactive state while preserving the integrity of the host cell. SCVs possess important metabolic and phenotypic differences from ordinary *S. aureus* isolates [94–96]. Indeed, they are relatively resistant to antibiotics [97,98] and, hence, difficult to eradicate with antibiotic therapy [99].

Moreover, the synthesis and secretion of glycocalyx play a role in the virulence of *S. aureus*. This is also documented for strains obtained from DFI [100]. The polysaccharide production begins immediately after the adhesion and covers the bacteria, representing an essential component for the development of a 'biofilm' (Figure 3) [101–104].

In addition to specific adherence mechanisms, *S. aureus* have a number of other virulence factors involved in the infection of soft tissues and bones. *S. aureus* is able to secrete toxins, which can lead to tissue necrosis (Figure 3). Toxins produced by *S. aureus* have an important role in the deepening and spread of the infection in the patient with DFI.
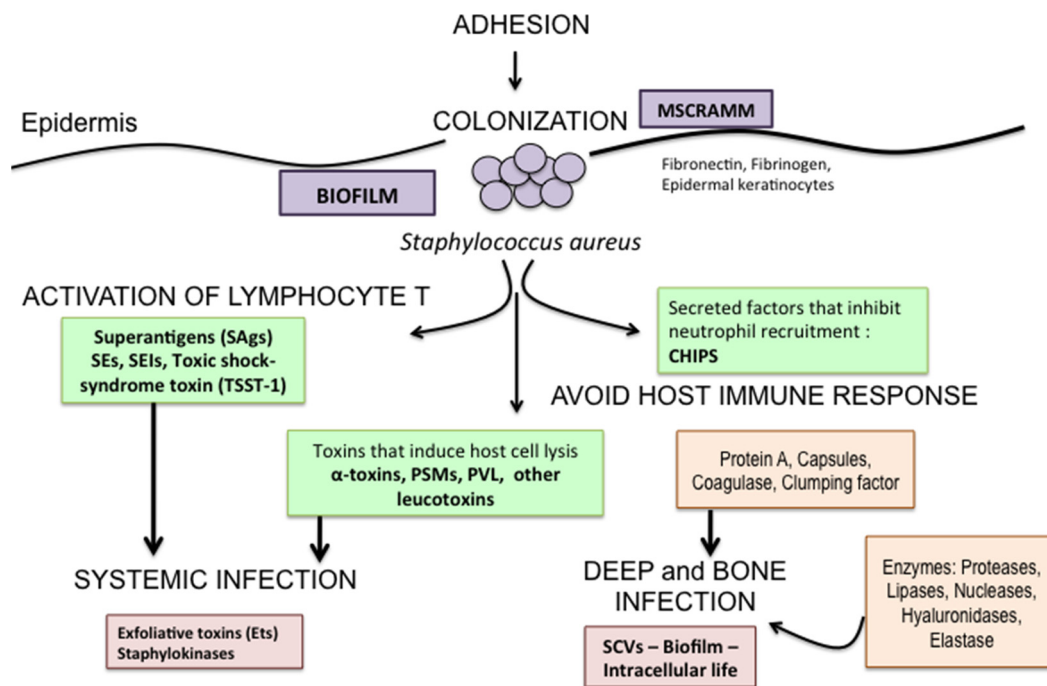
Figure 3. Infection process and virulence factors of *Staphylococcus aureus* in diabetic foot infection; MSCRAMM: microbial surface components recognizing adhesive matrix molecules. *S. aureus* secretes several virulence factors that evade host immune defenses. α-hemolysin (or α-toxin), phenol-soluble modulins (PSMs) and Panton-Valentine leukocidin (PVL) have the capacity to lyse host cells, which is a mechanism to evade immune responses. In addition, *S. aureus* secretes factors that inhibit neutrophil function, such as chemotaxis inhibitory protein of staphylococci (CHIPS). In addition, *S. aureus* possesses factors that active lymphocyte T cells: the superantigens (SE: enterotoxins; SEI: enterotoxin-like protein; TSST: toxic shock-syndrome toxin). On the other side, exfoliative toxins (Ets) facilitate bacterial skin invasion.

3. *Staphylococcus aureus* Toxins in DFIs

The ability of *S. aureus* to cause DFI is defined by numerous virulence factors among which secreted toxins play an important role (participation in colonization, persistence, evasion of the immune system and dissemination) [105]. These toxins include: the pore-forming toxins, the exfoliatins, the superantigen exotoxins (SAg) and the EDIN (epidermal cell differentiation inhibitors) toxins. These cytolytic toxins can damage membranes of host cells leading to cell lysis [106]. Hemolysins lyse red blood cells, while leukotoxins target white blood cells.

3.1. Pore-Forming Toxins

Pore-forming toxins (PFT) of *S. aureus*, through pore-forming and pro-inflammatory activities, have the ability to lyse host cells. They include the single-component α-toxin (or α-hemolysin), the phenol-soluble modulins (PSMs) and bi-component leukotoxins, including Panton-Valentine leukocidin (PVL), γ-hemolysin and leukocidin D/E [86].

3.1.1. α-Toxin

This PFT is a beta-barrel forming toxin, which consists of beta sheets [107]. It is released by the majority of *S. aureus* as a water-soluble monomer [108]. Its targets are red blood cells and leukocytes except neutrophils [109]. Although α-toxin is the most frequently secreted, few studies have focused on the role of this hemolysin produced by *S. aureus* in DFI. In a French national study, almost all of the strains harbored the α-toxin-encoding gene *hla* independently of the grade [110]. However,

this proportion varies between methicillin-susceptible *S. aureus* (MSSA) and MRSA. The α-hemolysin gene was significantly less present in MRSA (16.4%) than in MSSA strains (100%) [48]. As we noted previously, DFIs caused by MRSA present a similar severity of infections to MSSA, excluding an increased role of α-toxin in the pathogenicity of MSSA.

3.1.2. Phenol Soluble Modulins

Recently, the role of a family of secreted peptides, the phenol-soluble modulins (PSMs), has been described in staphylococcal pathogenesis [111]. PSMs are produced by the majority of *S. aureus* strains [112]. These toxins are membrane-injuring toxins. They are structurally characterized as a family of seven small amphipathic α-helical peptides. Some PSMs are described: PSMα1–PSMα4 and delta-toxin. Like LukAB (described below), they induce human neutrophil lysis after phagocytosis, a pathogenesis mechanism of great importance for the high toxicity [113,114]. In DFI, to date, no report has evaluated the significance of these virulence factors in the pathogenicity of *S. aureus*.

3.1.3. The Bi-Component Leukotoxins

S. aureus produces some bi-component toxins structurally similar to α-toxin. These toxins result from the association of the class S (Slow) component and the class F (Fast) component based on their electrophoretic mobility [105]. They induce the activation and the permeability of the target cells. They can lyse phagocytes (monocytes-macrophages and neutrophils), which is considered important for *S. aureus* immune evasion [115,116]. These PFTs include: (i) the gamma-toxin (gamma-hemolysins HlgA and HlgC/HlgB); (ii) the Panton-Valentine leukocidin (PVL) [117], corresponding to the LukS-PV and LukF-PV proteins; (iii) the leukocidins LukDE [118,119]; and LukAB [116] (also known as LukGH) [116].

The *hlg* gene cluster encoding for hemolysin-γ (Hlg) and hemolysin-γ2 (Hlg2) is located in the core genome. This cluster is present in almost all *S. aureus* strains. These toxins play a role in septic arthritis and could help community-acquired MRSA (CA-MRSA) to survive in human blood during infection [120,121]. In DFI, all of the isolated strains possess the different components of the *hlg* gene [48,110]. Interestingly, a variant of *hlg* (*hlgv*) is significantly associated with strains isolated from infected ulcers (Grades 2–4) [110,122].

The most well-known leukotoxin is PVL. PVL confers cytotoxicity on neutrophils and monocytes-macrophages, leading to a high virulence [123]. LukS-/LukF-PV are encoded within lysogenic phages [124,125]. Several *lukS-/lukF-PV*-transducing phages have been discovered [105]. This particular genetic organization involves an easy horizontal transmission of PVL genes in *Staphylococcus* spp. [126].

The PVL-positive strains are responsible for SSTIs (abscesses, furuncles, carbuncles or necrotizing fasciitis), severe necrotizing pneumonia and aggressive bone and joint infections [48,105,127,128]. However their prevalence is extremely diverse, varying between less than 5% and 67% of MSSA [129–136]. Interestingly, PVL-positive strains are statistically associated with younger patients [137]. This toxin has been linked to CA-MRSA infections [129], even if some CA-MRSA isolates do not carry the *lukS/lukF-PV* genes [138]. A high prevalence of CA-MRSA was observed in Africa [139], the Middle East [140,141], Asia [142] and America [143].

The PVL-producing clones were rarely isolated from DFI (Table 2) [48,110,122,144,145]. However, this prevalence varies between countries: France (~3%), Algeria and The Netherlands (~14%) [48,146]. The main PVL-producing strains isolated from DFI belong to ST80-MRSA (14/21 strains), followed by ST152 (6/21) and CC30 (1/21). ST80 is the main PVL clone circulating in Europe and North Africa [147–149]. Rates of PVL-producing clones in DFI remain low in comparison to data concerning SSTIs [127] (e.g., the epidemic furuncles (42%), major abscesses (73%) and gold surgically-drained abscesses (89%)) [146,150–152]. Indeed, in DFI, a higher incidence of PVL-positive isolates among subjects with CA-MRSA could be observed (31.8% versus 5.7%; *p* = 0.004) [153].

Table 2. Repartition of toxin-encoding genes in *Staphylococcus aureus* isolated from diabetic foot ulcers at different clinical grades.

Genes	PEDIS Grades			Total	References
	Grade 1	Grades 2–3	Grade 4		
	n = 99	*n* = 481	*n* = 94	*n* = 674	
lukF/luS-PV	3 (3%)	15 (3.1%)	3 (3.2%)	21 (3.1%)	[48,110,122,145]
edin	6 (6.1%)	12 (2.5%)	10 (10.6%)	28 (4.1%)	[48,110,122,145]
tsst	5 (5.1%)	40 (8.3%)	12 (12.8%)	57 (8.5%)	[48,110,122,145]
etA, etB, etD	4 (4.0%)	17 (3.5%)	13 (13.8%)	34 (5.0%)	[48,110,122,145]
sea	39 (39.4%)	201 (41.8%)	39 (41.5%)	279 (41.4%)	[48,110,122,145]
lukDE	66 (66.7%)	263 (54.7%)	49 (52.1%)	378 (56.1%)	[48,110,122,145]

The role of PVL in DFI remains under debate. The different PVL clones are equally distributed among each grade [122]. The majority of Grade 1 ulcers where PVL-positive strains were isolated had a rapid amelioration [122], and for instance, in Algeria, all of the patients harboring PVL-positive strains isolated from DFI had a worsening evolution. However, the management of chronic wound infections in this country is clearly different from international recommendations, and the use of amputations is frequent, independent of the evolution of the wound. Finally, it is interesting to note that strains isolated from DFOM harbor neither *lukF-* nor *lukS-PV* [154]. Thus PVL-positive strains are scarce in wound ulcers, and their pathogenicity is not clearly established.

LukED exhibits toxicity toward PMNs in vitro and induces dermatonecrosis when purified toxin is injected into rabbits [118,119]. Moreover, this toxin plays a critical role in *S. aureus* lethality for mice. It targets and kills murine phagocytes (monocytes-macrophages and neutrophils), promoting disease progression [155]. In uninfected DFU, LukED is equally distributed among grades (52%–66%) when we pooled all of the data (Table 2). However, when data are analyzed separately, we showed that the *lukDE* gene was significantly more often associated with strains isolated from infected ulcers (Grades 2–4) [122]. In a more recent study, this gene was clearly identified as a marker that differentiated uninfected from infected ulcers and predicted the outcome of Grade 1 DFU [110]. The association between *lukDE* and MRSA has been also reported in DFI [48,156]. In DFOM, this gene was present in approximately 40% of strains [154]. However, even if experimental analysis showed the virulence potential of this leukotoxin, clinically, it seems to present a poorer activity compared to PVL, and the reduced virulence observed may be a response to atypical local inflammatory reaction [156].

The last leukocidin characterized is LukAB/HG. This new member of the *S. aureus* leukotoxin family contributes to neutrophil killing, promotes the survival of *S. aureus* in human whole blood, restricts neutrophil-mediated killing and promotes CA-MRSA pathogenesis [115,116]. No data concerning its implication in DFU have been reported.

3.2. Exfoliative Toxins

Exfoliative toxins are serine proteases secreted by *S. aureus*. Three (ETA, ETB and ETD) out of the four different serotypes of this toxin are linked to human infection. The exfoliatins act as "molecular scissors" facilitating bacterial skin invasion [157]. The prevalence of *eta* and/or *etb* ranges from 0.5%–3% in MSSA [157–159], whereas around 10% of MRSA are *eta*-positive [159].

In uninfected DFU, the distribution varies among clinical grades (Table 2). Interestingly, strains harboring these genes are three-times more frequent in Grade 4 (13.8%) as compared to Grade 1 (4%) or Grades 2–3 (3.5%). However, each exfoliatin does not have the same representation: if *eta* and *etb* are rare (1.3%) or absent, respectively, *etd* is the most prevalent (3.7%), particularly in strains isolated from Grade 4 (10.6%). In a previous study, we could also note that two of four patients harboring an *etd*-positive strain present on a Grade 1 ulcer had a worsening evolution [17]. Post et al. showed an important presence of *eta* (13%) and *etb* (17%) in DFI (no screening of *etD* was noted) [144]. However,

as no grade has been reported in this work, it is not possible to link exfoliative genes and the severity of the DFI. Finally, this gene was absent in strains isolated from DFOM [154].

3.3. Enterotoxins

Enterotoxins are secreted toxins of ~20–30 kD that belong to the family of superantigens (SAg). These molecules over-induce cytokine production from both T-lymphocytes and macrophages [107]. The mechanisms by which staphylococcal enterotoxins work are not well known, but may include the activation of cytokine release, ultimately causing cell death by apoptosis. They contribute significantly to major illnesses [160,161]. A recent classification distinguishes three groups of SAg: staphylococcal enterotoxins (SEs), staphylococcal enterotoxin-like toxins (SEls) and toxic shock-syndrome toxin 1 (TSST-1) [162].

3.3.1. Staphylococcal Enterotoxins and Enterotoxin-Like Toxins

The majority of *S. aureus* isolated from DFU have the capacity to produce a large number of Sags, notably SEs and SEls [161]. These toxins activate T cells, resulting in a high secretion of proinflammatory cytokines. This process leads to a chronic inflammatory state in uninfected DFUs, inducing a delay or an absence of wound healing [163]. Genes, including the *sea*, *sed*, *seg* and *sei* genes, code for enterotoxins found in *S. aureus* isolated from DFI. We observed that *sea* and *sei* are significantly more prevalent in Grade 2–4 ulcers than in Grade 1 (Table 2) [122] and could represent a biomarker to differentiate infection and colonization. The majority of enterotoxins are more frequently identified in MRSA strains except for *seb* and *seh* genes [122]. One of the main enterotoxins is SED, present in around 40% of the strains [122,161]. The *sed* gene is often located on a plasmid, and the active protein is structurally similar to SEA [164]. SEA could have a major role in atopic dermatitis by inducing the upregulation of adhesion molecules and eliciting inflammatory responses in endothelial cells and keratinocytes [165]. Thus, SED may be selected in DFU isolates because, similar to SEA, it has an enhanced ability to induce local inflammatory responses.

3.3.2. Toxic Shock-Syndrome Toxin 1

The best known *S. aureus* superantigen is the 22-kD toxic shock-syndrome toxin 1 (TSST-1), which causes toxic shock syndrome (TSS). Additionally, SEl-X is a new member of the *S. aureus* SAg family, and it has been shown to have an important role in *S. aureus* necrotizing pneumonia infection caused by USA300 MRSA strain [166]. Vu et al. found that 88% of the DFU isolates carried the gene for SEl-X; the remainder contained the gene for TSST-1, and one isolate had genes for both SEl-X and TSST-1. Typically, *S. aureus* strains have the gene for either SEl-X or TSST-1 [161]. The prevalence of the *tsst* gene is low in the strains isolated from diabetic foot (~8%). However, as we observed for *etD*, this gene is more frequently present in Grade 4 (Table 2) and absent in DFOM [154].

3.4. Epidermal Cell Differentiation Inhibitors Toxins

EDINs toxins are members of a group of major bacterial virulence factors targeting host Rho GTPases [167]. Recent findings suggest that EDIN toxins might favor bacterial dissemination in tissues by a hematogenous route, through the induction of large transcellular tunnels in endothelial cells named macroapertures [168–170]. Indeed, recent data showed that EDIN toxins promote the formation of infection foci in a mouse model of bacteremia [171]. To date, three isoforms of EDIN have been characterized: the first discovered EDIN isoform (EDIN-A), isolated from the E-1 strain of *S. aureus* [172], as well as EDIN-B [173,174] and EDIN-C [175]. A first epidemiological survey, involving staphylococcal strains isolated from patients hospitalized for various infectious diseases, demonstrated a higher prevalence of *edin*-encoding genes in this group compared to nasal strains isolated from healthy patients [176]. Munro et al. showed that 90% of all *edin*-bearing *S. aureus* isolates carry the type-C allele. These isolates are more significantly associated with deep-seated soft tissue infections than other types of infections [177].

Messad et al. analyzed the distribution of *edin* genes in *S. aureus* isolated from DFI in a French national collection. *edin-B* is the most prevalent *edin* gene associated with DFIs (Table 2) [17]. The clonal complex analysis indicated that *edin*-positive strains belonged to four major groups: a singleton near CC8 (*edin-A*), a singleton belonging to ST152-MSSA (*edin-B*), CC80-MRSA (*edin-B*) and the most prevalent CC25/28-MSSA (*edin-B*). The distribution of *edin* genes in DFI shows an important presence of these genes in strains isolated from Grade 4 ulcers (2.5% for Grades 2–3 vs. 10.6% for Grade 4). Of note, patients with Grade 1 ulcers that presented *edin*-positive strains had a worsening evolution [17]. However, these genes seem to be absent in strains isolated from DFOM [154]. These observations support the idea that EDIN might work together with the arsenal of *S aureus* virulence factors to give the bacteria a higher potential for systemic infection [168]. *edin* encoding genes thus represent additional markers of interest to differentiate infecting from colonizing *S. aureus* strains in DFU and to predict the wound outcome.

4. Conclusions

In conclusion, DFI is a complex pathology involving the virulence of bacteria and host responses. Indeed, its main feature is the coexistence of multiple bacterial species on the chronic wound organized in pathogroups and the host-related responses encountered by bacteria, which modify the bacterial pathogenicity. Some studies showed the presence of toxinogenic *S. aureus* strains (harboring exfoliatins-, EDIN-, PVL- or TSST-encoding genes) in DFI, notably in Grade 4, with systemic impact. The absence of the same strains in DFOM suggests that these strains may not be adapted to colonize wounds. On the other hand, the main population of *S. aureus* (non-toxinogenic) isolated in uninfected DFU is perfectly adapted to infect deep structures and bone. The rarity of systemic cases (Grade 4) and of the presence of toxinogenic bacteria would, by definition, be an infected wound. However, it is interesting to note that some toxinogenic strains with a high pathogenic potential are present on uninfected wounds promoting no virulence in this polymicrobial environment. Moreover, no clear link between these strains and amputations has been noted to date. Subsequent studies are required to understand the role of toxins and their real impact in DFU. Screening the presence of genes encoding toxins by molecular biology tests on uninfected DFU could also represent a new approach for patients for whom the clinical diagnosis of infection is hampered by peripheral arterial disease, neuropathy or impaired leukocyte functions in the aim to predict if *S. aureus* is going to be invasive or not (needing an antibiotic treatment).

Author Contributions: C.D.-R., C.N.E., A.S. and J.-P.L wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Singh, N.; Armstrong, D.G.; Lipsky, B.A. Preventing foot ulcers in patients with diabetes. *JAMA* **2005**, *293*, 217–228. [CrossRef] [PubMed]
2. Prompers, L.; Huijberts, M.; Schaper, N.; Apelqvist, J.; Bakker, K.; Edmonds, M.; Holstein, P.; Jude, E.; Jirkovska, A.; Mauricio, D.; et al. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. *Diabetologia* **2008**, *51*, 1826–1834. [CrossRef] [PubMed]
3. Lipsky, B.A.; Berendt, A.R.; Deery, H.G.; Embil, J.M.; Joseph, W.S.; Karchmer, A.W.; LeFrock, J.L.; Lew, D.P.; Mader, J.T.; Norden, C.; et al. Diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis.* **2004**, *39*, 885–910. [CrossRef] [PubMed]
4. Lavery, L.A.; Armstrong, D.G.; Wunderlich, R.P.; Tredwell, J.; Boulton, A.J. Diabetic foot syndrome: Evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. *Diabetes Care* **2003**, *26*, 1435–1438. [CrossRef] [PubMed]

5. Jeffcoate, W.J.; Lipsky, B.A.; Berendt, A.R.; Cavanagh, P.R.; Bus, S.A.; Peters, E.J.; van Houtum, W.H.; Valk, G.; Bakker, K. Unresolved issues in the management of ulcers of the foot in diabetes. *Diabet. Med.* **2008**, *25*, 1380–1389. [CrossRef] [PubMed]

6. Lavery, L.A.; Armstrong, D.G.; Wunderlich, R.P.; Mohler, M.J.; Wendel, C.S.; Lipsky, B.A. Risk factors for foot infections in individuals with diabetes. *Diabetes Care* **2006**, *29*, 1288–1293. [CrossRef] [PubMed]

7. Richard, J.L.; Lavigne, J.P.; Sotto, A. Diabetes and foot infection: More than double trouble. *Diabetes Metab. Res. Rev.* **2012**, *28*, 46–53. [CrossRef] [PubMed]

8. Spichler, A.; Hurwitz, B.L.; Armstrong, D.G.; Lipsky, B.A. Microbiology of diabetic foot infections: From Louis Pasteur to "crime scene investigation". *BMC Med.* **2015**, *13*, 2. [CrossRef] [PubMed]

9. Lavigne, J.P.; Sotto, A.; Dunyach-Remy, C.; Lipsky, B.A. New molecular techniques to study the skin microbiota of diabetic foot ulcers. *Adv. Wound Care* **2015**, *4*, 38–49. [CrossRef] [PubMed]

10. Edmonds, M. Infection in the neuroischemic foot. *Int. J. Low. Extrem. Wounds* **2005**, *4*, 145–153. [CrossRef] [PubMed]

11. Williams, D.T.; Hilton, J.R.; Harding, K.G. Diagnosing foot infection in diabetes. *Clin. Infect. Dis.* **2004**, *39*, S83–S86. [CrossRef] [PubMed]

12. Armstrong, D.G.; Lavery, L.A.; Sariaya, M.; Ashry, H. Leukocytosis is a poor indicator of acute osteomyelitis of the foot in diabetes mellitus. *J. Foot Ankle Surg.* **1996**, *35*, 280–283. [CrossRef]

13. Eneroth, M.; Larsson, J.; Apelqvist, J. Deep foot infections in patients with diabetes and foot ulcer: An entity with different characteristics, treatments, and prognosis. *J. Diabetes Complicat.* **1999**, *13*, 254–263. [CrossRef]

14. Eneroth, M.; Apelqvist, J.; Stenström, A. Clinical characteristics and outcome in 223 diabetic patients with deep foot infections. *Foot Ankle Int.* **1997**, *18*, 716–722. [CrossRef] [PubMed]

15. Lipsky, B.A. International consensus group on diagnosing and treating the infected diabetic foot. A report from the international consensus on diagnosing and treating the infected diabetic foot. *Diabetes Metab. Res. Rev.* **2004**, *20*, S68–S77. [CrossRef] [PubMed]

16. Richard, J.L.; Sotto, A.; Lavigne, J.P. New insights in diabetic foot infection. *World J. Diabetes* **2011**, *15*, 24–32. [CrossRef] [PubMed]

17. Messad, N.; Landraud, L.; Canivet, B.; Lina, G.; Richard, J.L.; Sotto, A.; Lavigne, J.P. Distribution of *edin* in *Staphylococcus aureus* isolated from diabetic foot ulcers. *Clin. Microbiol. Infect.* **2013**, *19*, 875–880. [CrossRef] [PubMed]

18. Gardner, S.E.; Hillis, S.L.; Heilmann, K.; Segre, J.A.; Grice, E.A. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes* **2013**, *62*, 923–930. [CrossRef] [PubMed]

19. Hatipoglu, M.; Mutluoglu, M.; Turhan, V.; Uzun, G.; Lipsky, B.A.; Turk-DAY Study Group; Sevim, E.; Demiraslan, H.; Eryilmaz, E.; Ozuguz, C.; et al. Causative pathogens and antibiotic resistance in diabetic foot infections: A prospective multi-center study. *J. Diabetes Complicat.* **2016**, *30*, 910–916. [CrossRef] [PubMed]

20. Commons, R.J.; Robinson, C.H.; Gawler, D.; Davis, J.S.; Price, R.N. High burden of diabetic foot infections in the top end of Australia: An emerging health crisis (DEFINE study). *Diabetes Res. Clin. Pract.* **2015**, *110*, 147–157. [CrossRef] [PubMed]

21. Lesens, O.; Desbiez, F.; Theïs, C.; Ferry, T.; Bensalem, M.; Laurichesse, H.; Tauveron, I.; Beytout, J.; Aragón-Sánchez, J.; Working Group on Diabetic Osteomyelitis. *Staphylococcus aureus*-related diabetic osteomyelitis: Medical or surgical management? A French and Spanish retrospective cohort. *Int. J. Low. Extrem. Wounds* **2015**, *14*, 284–290. [CrossRef] [PubMed]

22. Serra, R.; Grande, R.; Butrico, L.; Rossi, A.; Settimio, U.F.; Caroleo, B.; Amato, B.; Gallelli, L.; de Franciscis, S. Chronic wound infections: The role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Expert Rev. Anti-Infect. Ther.* **2015**, *13*, 605–613. [CrossRef] [PubMed]

23. Wertheim, H.F.; Melles, D.C.; Vos, M.C.; van Leeuwen, W.; van Belkum, A.; Verbrugh, H.A.; Nouwen, J.L. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* **2005**, *5*, 751–762. [CrossRef]

24. Tong, S.Y.; David, J.S.; Eichenberger, E.; Holland, T.L.; Fowler, V.G., Jr. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev.* **2015**, *28*, 603–661. [CrossRef] [PubMed]

25. Lipsky, B.A.; Aragon-Sanchez, J.; Embil, J.; Kono, S.; Lavery, L.; Senneville, E.; Urbancic-Rovan, V.; Van Asten, S.; Peters, E.J.G.; International Working Group on the Diabetic Foot (IWGDF). IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. *Diabetes Metab. Res. Rev.* **2016**, *32*, 45–74. [CrossRef] [PubMed]

26. Shaper, N.C. Diabetic foot ulcer classification system for research purposes: A progress report on criteria for including patients in research studies. *Diabetes Metab. Res. Rev.* **2004**, *20*, S90–S95. [CrossRef] [PubMed]

27. Lipsky, B.A.; Berendt, A.R.; Cornia, P.B.; Pile, J.C.; Peters, E.J.; Armstrong, D.G.; Deery, H.G.; Embil, J.M.; Joseph, W.S.; Karchmer, A.W.; et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *J. Am. Podiatr. Med. Assoc.* **2013**, *103*, 2–7. [CrossRef] [PubMed]

28. Lipsky, B.A. Osteomyelitis of the foot in diabetic patients. *Clin. Infect. Dis.* **1997**, *25*, 1318–1326. [CrossRef] [PubMed]

29. Markanday, A. Diagnosing diabetic foot osteomyelitis: Narrative review and a suggested 2-step score-based diagnostic pathway for clinicians. *Open Forum Infect. Dis.* **2014**, *1*, ofu060. [CrossRef] [PubMed]

30. Newman, L.G.; Waller, J.; Palestro, C.J.; Schwartz, M.; Klein, M.J.; Hermann, G.; Harrington, E.; Harrington, M.; Roman, S.H.; Stagnaro-Green, A. Unsuspected osteomyelitis in diabetic foot ulcers. Diagnosis and monitoring by leukocyte scanning with indium in 111 oxyquinoline. *JAMA* **1991**, *266*, 1246–1251. [CrossRef] [PubMed]

31. Lazaro, J.L.; Izzo, V.; Meaume, S.; Davies, A.H.; Lobmann, R.; Uccioli, L. Elevated levels of matrix metalloproteinases and chronic wound healing: An updated review of clinical evidence. *J. Wound Care* **2016**, *25*, 277–287. [CrossRef] [PubMed]

32. McCarty, S.M.; Cochrane, C.A.; Clegg, P.D.; Percival, S.L. The role of endogenous and exogenous enzymes in chronic wounds: A focus on the implications of aberrant levels of both host and bacterial proteases in wound healing. *Wound Repair Regen.* **2012**, *20*, 125–136. [CrossRef] [PubMed]

33. Ren, Y.; Gu, G.; Yao, M.; Driver, V.R. Role of matrix metalloproteinases in chronic wound healing: Diagnostic and therapeutic implications. *Chin. Med. J.* **2014**, *127*, 1572–1581. [PubMed]

34. Chang, M. Restructuring of the extracellular matrix in diabetic wounds and healing: A perspective. *Pharmacol. Res.* **2016**, *107*, 243–248. [CrossRef] [PubMed]

35. Visse, R.; Nagase, H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. *Circ. Res.* **2003**, *92*, 827–839. [CrossRef] [PubMed]

36. Li, Z.; Guo, S.; Yao, F.; Zhang, Y.; Li, T. Increased ratio of serum matrix metalloproteinase-9 against TIMP-1 predicts poor wound healing in diabetic foot ulcers. *J. Diabetes Complicat.* **2013**, *27*, 380–382. [CrossRef] [PubMed]

37. Yager, D.R.; Zhang, L.Y.; Liang, H.X.; Diegelmann, R.F.; Cohen, I.K. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J. Investig. Dermatol.* **1996**, *107*, 743–748. [CrossRef] [PubMed]

38. Saito, S.; Trovato, M.J.; You, R.; Lal, B.K.; Fasehun, F.; Padberg, F.T., Jr.; Hobson, R.W., 2nd; Durán, W.N.; Pappas, P.J. Role of matrix metalloproteinases 1, 2, and 9 and tissue inhibitor of matrix metalloproteinase-1 in chronic venous insufficiency. *J. Vasc. Surg.* **2001**, *34*, 930–938. [CrossRef] [PubMed]

39. Lobmann, R.; Ambrosch, A.; Schultz, G.; Waldmann, K.; Schiweck, S.; Lehnert, H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia* **2002**, *45*, 1011–1016. [CrossRef] [PubMed]

40. Grinnell, F.; Zhu, M. Fibronectin degradation in chronic wounds depends on the relative levels of elastase, alpha1-proteinase inhibitor, and alpha2-macroglobulin. *J. Investig. Dermatol.* **1996**, *106*, 335–341. [CrossRef] [PubMed]

41. Tentolouris, N.; Petrikkos, G.; Vallianou, N.; Zachos, C.; Daikos, G.L.; Tsapogas, P.; Markou, G.; Katsilambros, N. Prevalence of methicillin-resistant *Staphylococcus aureus* in infected and uninfected diabetic foot ulcers. *Clin. Microbiol. Infect.* **2006**, *12*, 186–189. [CrossRef] [PubMed]

42. Game, F.; Jeffcoate, W. MRSA and osteomyelitis of the foot in diabetes. *Diabet. Med.* **2004**, *21*, 16–19. [CrossRef] [PubMed]

43. Dang, C.N.; Prasad, Y.D.; Boulton, A.J.; Jude, E.B. Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: A worsening problem. *Diabet. Med.* **2003**, *20*, 159–161. [CrossRef] [PubMed]

44. Barshes, N.R.; Rodriguez-Barradas, M.C.; Bechara, C.F.; Pisimisis, G.; Young, E.J.; Kougias, P. Microbial isolates and their antimicrobial susceptibilities in inframalleolar foot infections. *Surg. Infect.* **2014**, *15*, 585–591. [CrossRef] [PubMed]

45. Citron, D.M.; Goldstein, E.J.; Merriam, C.V.; Lipsky, B.A.; Abramson, M.A. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J. Clin. Microbiol.* **2007**, *45*, 2819–2828. [CrossRef] [PubMed]

46. Lavery, L.A.; Harkless, L.B.; Felder-Johnson, K.; Mundine, S. Bacterial pathogens in infected puncture wounds in adults with diabetes. *J. Foot Ankle Surg.* **1994**, *33*, 91–97. [PubMed]

47. Lavery, L.A.; Sariaya, M.; Ashry, H.; Harkless, L.B. Microbiology of osteomyelitis in diabetic foot infections. *J. Foot Ankle Surg.* **1995**, *34*, 61–64. [CrossRef]

48. Djahmi, N.; Messad, N.; Nedjai, S.; Moussaoui, A.; Mazouz, D.; Richard, J.L.; Sotto, A.; Lavigne, J.P. Molecular epidemiology of *Staphylococcus aureus* strains isolated from inpatients with infected diabetic foot ulcers in an Algerian University Hospital. *Clin. Microb. Infect.* **2013**, *19*, E398–E404. [CrossRef] [PubMed]

49. Richard, J.L.; Lavigne, J.P.; Got, I.; Hartemann, A.; Malgrange, D.; Tsirtsikolou, D.; Baleydier, A.; Senneville, E. Management of patients hospitalized for diabetic foot infection: Results of the French OPIDIA study. *Diabetes Metab.* **2011**, *37*, 208–215. [CrossRef] [PubMed]

50. Al Benwan, K.; Al Mulla, A.; Rotimi, V.O. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwai. *J. Infect. Public Health* **2012**, *5*, 1–8. [CrossRef] [PubMed]

51. Akhi, M.T.; Ghotaslou, R.; Asgharzadeh, M.; Varshochi, M.; Pirzadeh, T.; Memar, M.Y.; Zahedi Bialvaei, A.; Seifi Yarijan Sofla, H.; Alizadeh, N. Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran. *GMS Hyg. Infect. Control* **2015**, *10*, Doc02. [CrossRef] [PubMed]

52. Anvarinejad, M.; Pouladfar, G.; Japoni, A.; Bolandparvaz, S.; Satiary, Z.; Abbasi, P.; Mardaneh, J. Isolation and antibiotic susceptibility of the microorganisms isolated from diabetic foot infections in Nemazee Hospital, Southern Iran. *J. Pathog.* **2015**, *2015*. [CrossRef] [PubMed]

53. Alavi, S.M.; Khosravi, A.D.; Sarami, A.; Dashtebozorg, A.; Montazeri, E.A. Bacteriologic study of diabetic foot ulcer. *Pak. J. Med. Sci.* **2007**, *235*, 681–684. [CrossRef]

54. Raja, N.S. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: A retrospective study of 194 cases. *J. Microbiol. Immunol. Infect.* **2007**, *40*, 39–44. [PubMed]

55. Yoga, R.; Khairul, A.; Sunita, K.; Suresh, C. Bacteriology of diabetic foot lesions. *Med. J. Malays.* **2006**, *61*, 14–16.

56. Martinez-Gomez Dde, A.; Ramirez-Almagro, C.; Campillo-Soto, A.; Morales-Cuenca, G.; Pagan-Ortiz, J.; Aguayo-Albasini, J.L. Diabetic foot infections. Prevalence and antibiotic sensitivity of the causative microorganisms. *Enferm. Infecc. Microbiol. Clin.* **2009**, *27*, 317–321. [PubMed]

57. Perim, M.C.; Bordes Jda, C.; Celeste, S.R.; Orsolin Ede, F.; Mendes, R.R.; Mendes, G.O.; Ferreira, R.L.; Carreiro, S.C.; Pranchevicius, M.C. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. *Rev. Soc. Bras. Med. Trop.* **2015**, *48*, 546–554. [CrossRef] [PubMed]

58. Shanmugam, P.; Jeya, M.; Linda Susan, S. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. *J. Clin. Diagn. Res.* **2013**, *7*, 441–445. [CrossRef] [PubMed]

59. Sugandhi, P.; Prasanth, D.A. Microbiological profile of bacterial pathogens from diabetic foot infections in tertiary care hospitals, Salem. *Diabetes Metab. Syndr.* **2014**, *8*, 129–132. [CrossRef] [PubMed]

60. Mathangi, T.; Prabhakaran, P.; Rayappan, F.; Tilton, F. Isolation, molecular characterization and antibiogram of bacteria isolated from DFU. *Int. J. Curr. Res. Acad. Rev.* **2013**, *1*, 17–25.

61. Sekhar, S.M.; Vyas, N.; Unnikrishnan, M.K.; Rodrigues, G.S.; Mukhopadhyay, C. Antimicrobial susceptibility pattern in diabetic foot ulcer: A pilot study. *Ann. Med. Health Sci. Res.* **2014**, *4*, 742–745. [CrossRef] [PubMed]

62. Wang, S.H.; Sun, Z.L.; Guo, Y.J.; Yang, B.Q.; Yuan, Y.; Wei, Q.; Ye, K.P. Meticillin-resistant *Staphylococcus aureus* isolated from foot ulcers in diabetic patients in a Chinese care hospital: Risk factors for infection and prevalence. *J. Med. Microbiol.* **2010**, *59*, 1219–1224. [CrossRef] [PubMed]

63. Ding, Q.; Li, D.Q.; Wang, P.H.; Chu, Y.J.; Meng, S.Y.; Sun, Q. Risk factors for infections of methicillin-resistant Staphylococci in diabetic foot patients. *Zhonghua Yi Xue Za Zhi* **2012**, *92*, 228–231.

64. Sharma, V.K.; Khadka, P.B.; Joshi, A.; Sharma, R. Common pathogens isolated in diabetic foot infection in Bir Hospital. *Kathmandu Univ. Med. J.* **2006**, *4*, 295–301.

65. Islam, S.; Cawich, S.O.; Budhooram, S.; Harnarayan, P.; Mahabir, V.; Ramsewak, S.; Naraynsingh, V. Microbial profile of diabetic foot infections in Trinidad and Tobago. *Prim. Care Diabetes* **2013**, *7*, 303–308. [CrossRef] [PubMed]

66. Mendes, J.J.; Marques-Costa, A.; Vilela, C.; Neves, J.; Candeias, N.; Cavaco-Silva, P.; Melo-Cristino, J. Clinical and bacteriological survey of diabetic foot infections in Lisbon. *Diabetes Res. Clin. Pract.* **2012**, *95*, 153–161. [CrossRef] [PubMed]

67. Radji, M.; Putri, C.S.; Fauziyah, S. Antibiotic therapy for diabetic foot infections in a tertiary care hospital in Jakarta, Indonesia. *Diabetes Metab. Syndr.* **2014**, *8*, 221–224. [CrossRef] [PubMed]

68. El-Tahawy, A.T. Bacteriology of diabetic foot. *Saudi Med. J.* **2000**, *21*, 344–347. [PubMed]

69. Guira, O.; Tiéno, H.; Traoré, S.; Diallo, I.; Ouangré, E.; Sagna, Y.; Zabsonré, J.; Yanogo, D.; Traoré, S.S.; Drabo, Y.J. The bacterial microflora of diabetic foot infection and factors determining its spectrum in Ouagadougou (Burkina Faso). *Bull. Soc. Pathol. Exot.* **2015**, *108*, 307–311. [CrossRef] [PubMed]

70. Unachukwu, C.N.; Obunge, O.K.; Odia, O.J. The bacteriology of diabetic foot ulcers in Port Harcourt, Nigeria. *Niger. J. Med.* **2005**, *14*, 173–176. [PubMed]

71. Katz, D.E.; Friedman, N.D.; Ostrovski, E.; Ravid, D.; Amrami, N.; Avivi, D.; Mengesha, B.; Zaidenstein, R.; Lazarovitch, T.; Dadon, M.; et al. Diabetic foot infection in hospitalized adults. *J. Infect. Chemother.* **2016**, *22*, 167–173. [CrossRef] [PubMed]

72. Cervantes-Garcia, E.; García-González, R.; Reséndiz-Albor, A.; Salazar-Schettino, P.M. Infections of diabetic foot ulcers with methicillin-resistant *Staphylococcus aureus*. *Int. J. Low. Extrem. Wounds* **2015**, *14*, 44–49. [CrossRef] [PubMed]

73. Lelievre, H.; Lina, G.; Jones, M.E.; Olive, C.; Forey, F.; Roussel-Delvallez, M.; Nicolas-Chanoine, M.H.; Bébéar, C.M.; Jarlier, V.; Andremont, A.; et al. Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. *J. Clin. Microbiol.* **1999**, *37*, 3452–3457. [PubMed]

74. Ertugrul, B.M.; Oncul, O.; Tulek, N.; Willke, A.; Sacar, S.; Tunccan, O.G.; Yilmaz, E.; Kaya, O.; Ozturk, B.; Turhan, O.; et al. A prospective, multi-center study: Factors related to the management of diabetic foot infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **2012**, *31*, 2345–2352. [CrossRef] [PubMed]

75. Leclercq, R. Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. *Clin. Microbiol. Infect.* **2009**, *15*, 224–231. [CrossRef] [PubMed]

76. Société de Pathologie Infectieuse de Langue Française. Management of diabetic foot infections. Short text. *Med. Mal. Infect.* **2007**, *37*, 1–25.

77. American Diabetes Association. Consensus development conference on diabetic foot wound care. *Diabetes Care* **1999**, *22*, 1354–1360.

78. Eleftheriadou, I.; Tentolouris, N.; Argiana, V.; Jude, E.; Boulton, A.J. Methicillin-resistant *Staphylococcus aureus* in diabetic foot infections. *Drugs* **2010**, *70*, 1785–1797. [CrossRef] [PubMed]

79. Lagacé-Wiens, P.R.; Ormiston, D.; Nicolle, L.E.; Hilderman, T.; Embil, J. The diabetic foot clinic: Not a significant source for acquisition of methicillin-resistant *Staphylococcus aureus*. *Am. J. Infect. Control* **2009**, *37*, 587–589. [CrossRef] [PubMed]

80. Pittet, D.; Hugonnet, S.; Harbarth, S.; Mourouga, P.; Sauvan, V.; Touveneau, S.; Perneger, T.V. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *Lancet* **2000**, *356*, 1307–1312. [CrossRef]

81. Zenelaj, B.; Bouvet, C.; Lipsky, B.A.; Uçkay, I. Do diabetic foot infections with methicillin-resistant *Staphylococcus aureus* differ from those with other pathogens? *Int. J. Low. Extrem. Wounds* **2014**, *13*, 263–272. [CrossRef] [PubMed]

82. Richard, J.L.; Sotto, A.; Jourdan, N.; Combescure, C.; Vannereau, D.; Rodier, M.; Lavigne, J.P. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. *Diabetes Metab.* **2008**, *34*, 363–369. [CrossRef] [PubMed]

83. Aragón-Sánchez, J.; Lázaro-Martínez, J.L.; Quintana-Marrero, Y.; Hernández-Herrero, M.J.; García-Morales, E.; Cabrera-Galván, J.J.; Beneit-Montesinos, J.V. Are diabetic foot ulcers complicated by MRSA osteomyelitis associated with worse prognosis? Outcomes of a surgical series. *Diabet. Med.* **2009**, *26*, 552–555. [CrossRef] [PubMed]

84. Chang, S.; Sievert, D.M.; Hageman, J.C.; Boulton, M.L.; Tenover, F.C.; Downes, F.P.; Shah, S.; Rudrik, J.T.; Pupp, G.R.; Brown, W.J.; et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. *N. Engl. J. Med.* **2003**, *348*, 1342–1347. [CrossRef] [PubMed]

85. Dezfulian, A.; Aslani, M.M.; Oskoui, M.; Farrokh, P.; Azimirad, M.; Dabiri, H.; Salehian, M.T.; Zali, M.R. Identification and characterization of a high vancomycin-resistant *Staphylococcus aureus* harboring *vanA* gene cluster isolated from diabetic foot ulcer. *Iran. J. Basic Med. Sci.* **2012**, *15*, 803–806. [PubMed]

86. Foster, T.J. Immune evasion by staphylococci. *Nat. Rev. Microbiol.* **2005**, *3*, 948–958. [CrossRef] [PubMed]

87. Ambrosch, A.; Haefner, S.; Jude, E.; Lobmann, R. Diabetic foot infections: Microbiological aspects, current and future antibiotic therapy focusing on methicillin-resistant *Staphylococcus aureus*. *Int. Wound J.* **2011**, *8*, 567–577. [CrossRef] [PubMed]

88. Cunningham, R.; Cockayne, A.; Humphreys, H. Clinical and molecular aspects of the pathogenesis of *Staphylococcus aureus* bone and joint infections. *J. Med. Microbiol.* **1996**, *44*, 157–164. [CrossRef] [PubMed]

89. Clarke, S.R.; Brummell, K.J.; Horsburgh, M.J.; McDowell, P.W.; Mohamad, S.A.; Stapleton, M.R.; Acevedo, J.; Read, R.C.; Day, N.P.; Peacock, S.J.; et al. Identification of in vivo-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. *J. Infect. Dis.* **2006**, *193*, 1098–1108. [CrossRef] [PubMed]

90. Weidenmaier, C.; Kokai-Kun, J.F.; Kristian, S.A.; Chanturiya, T.; Kalbacher, H.; Gross, M.; Nicholson, G.; Neumeister, B.; Mond, J.J.; Peschel, A. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat. Med.* **2004**, *10*, 243–245. [CrossRef] [PubMed]

91. Burian, M.; Rautenberg, M.; Kohler, T.; Fritz, M.; Krismer, B.; Unger, C.; Hoffmann, W.H.; Peschel, A.; Wolz, C.; Goerke, C. Temporal expression of adhesion factors and activity of global regulators during establishment of *Staphylococcus aureus* nasal colonization. *J. Infect. Dis.* **2010**, *201*, 1414–1421. [CrossRef] [PubMed]

92. Cho, S.H.; Strickland, I.; Boguniewicz, M.; Leung, D.Y. Fibronectin and fibrinogen contribute to the enhanced binding of *Staphylococcus aureus* to atopic skin. *J. Allergy Clin. Immunol.* **2001**, *108*, 269–274. [CrossRef] [PubMed]

93. Shi, S.; Zhang, X. Interaction of *Staphylococcus aureus* with osteoblasts. *Exp. Ther. Med.* **2012**, *3*, 367–370. [PubMed]

94. Proctor, R.A.; von Eiff, C.; Kahl, B.C.; Becker, K.; McNamara, P.; Herrmann, M.; Peters, G. Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat. Rev. Microbiol.* **2006**, *4*, 295–305. [CrossRef] [PubMed]

95. Tuchscherr, L.; Heitmann, V.; Hussain, M.; Viemann, D.; Roth, J.; von Eiff, C.; Peters, G.; Becker, K.; Löffler, B. *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. *J. Infect. Dis.* **2010**, *202*, 1031–1040. [CrossRef] [PubMed]

96. Von Eiff, C.; Becker, K.; Metze, D.; Lubritz, G.; Hockmann, J.; Schwarz, T.; Peters, G. Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: A cause for antibiotic treatment failure in a patient with darier's disease. *Clin. Infect. Dis.* **2001**, *32*, 1643–1647. [CrossRef] [PubMed]

97. Baumert, N.; von Eiff, C.; Schaaff, F.; Peters, G.; Proctor, R.A.; Sahl, H.G. Physiology and antibiotic susceptibility of *Staphylococcus aureus* small colony variants. *Microb. Drug Res.* **2002**, *8*, 253–260. [CrossRef] [PubMed]

98. Garcia, L.G.; Lemaire, S.; Kahl, B.C.; Becker, K.; Proctor, R.A.; Denis, O.; Tulkens, P.M.; Van Bambeke, F. Antibiotic activity against small-colony variants of *Staphylococcus aureus*: Review of in vitro, animal and clinical data. *J. Antimicrob. Chemother.* **2013**, *68*, 1455–1464. [CrossRef] [PubMed]

99. Proctor, R.A.; Peters, G. Small colony variants in staphylococcal infections: Diagnostic and therapeutic implications. *Clin. Infect. Dis.* **1998**, *27*, 419–422. [CrossRef] [PubMed]

100. Podbielska, A.; Galbowska, H.; Stelmach, E.; Mlynarczyk, G.; Olszewski, W.L. Slime production by *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from patients with diabetic foot ulcers. *Arch. Immunol. Ther. Exp.* **2010**, *58*, 321–324. [CrossRef] [PubMed]

101. Bergamini, T.M.; Corpus, R.A.; Hoeg, K.L.; Brittian, K.R.; Peyton, J.C.; Cheadle, W.G. Immune regulation of bacterial biofilm graft infection. *ASAIO J.* **1994**, *40*, 219–226. [CrossRef] [PubMed]

102. Steinberg, D.; Poran, S.; Shapira, L. The effect of extracellular polysaccharides from *Streptococcus mutans* on the bactericidal activity of human neutrophils. *Arch. Oral Biol.* **1999**, *44*, 437–444. [CrossRef]

103. Yasuda, H.; Ajiki, Y.; Aoyama, J.; Yokota, T. Interaction between human polymorphonuclear leucocytes and bacteria released from in vitro bacterial biofilm models. *J. Med. Microbiol.* **1994**, *41*, 359–367. [CrossRef] [PubMed]

104. Bjarnsholt, T.; Kirketerp-Moller, K.; Jensen, P.O.; Madsen, K.G.; Philipps, R.; Krogfelt, K.; Hoiby, N.; Givskov, M. Why chronic wounds will not heal: A novel hypothesis. *Wound Repair. Regen.* **2008**, *16*, 2–10. [CrossRef] [PubMed]

105. Vandenesch, F.; Lina, G.; Henry, T. *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors? *Front. Cell. Infect. Microbiol.* **2012**, *2*, 12. [CrossRef] [PubMed]

106. Grumann, D.; Nubel, U.; Broker, B.M. *Staphylococcus aureus* toxins—Their functions and genetics. *Infect. Genet. Evol.* **2014**, *21*, 583–592. [CrossRef] [PubMed]

107. Otto, M. *Staphylococcus aureus* toxins. *Curr. Opin. Microbiol.* **2014**, *17*, 32–37. [CrossRef] [PubMed]

108. Xiong, Y.Q.; Willard, J.; Yeaman, M.R.; Cheung, A.L.; Bayer, A.S. Regulation of *Staphylococcus aureus* alpha-toxin gene (*hla*) expression by *agr*, *sarA*, and *sae* in vitro and in experimental infective endocarditis. *J. Infect. Dis.* **2006**, *194*, 1267–1275. [CrossRef] [PubMed]

109. Valeva, A.; Walev, I.; Pinkernell, M.; Walker, B.; Bayley, H.; Palmer, M.; Bhakdi, S. Transmembrane beta-barrel of staphylococcal alpha-toxin forms in sensitive but not in resistant cells. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11607–11611. [CrossRef] [PubMed]

110. Sotto, A.; Richard, J.L.; Messad, N.; Molinari, N.; Jourdan, N.; Schuldiner, S.; Sultan, A.; Carrière, C.; Canivet, B.; Landraud, L.; et al. Distinguishing colonization from infection with *Staphylococcus aureus* in diabetic foot ulcers with miniaturized oligonucleotide arrays: A French multicenter study. *Diabetes Care* **2012**, *35*, 617–623. [CrossRef] [PubMed]

111. Wang, R.; Braughton, K.R.; Kretschmer, D.; Bach, T.H.; Queck, S.Y.; Li, M.; Kennedy, A.D.; Dorward, D.W.; Klebanoff, S.J.; Peschel, A.; et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat. Med.* **2007**, *13*, 1510–1514. [CrossRef] [PubMed]

112. Li, M.; Diep, B.A.; Villaruz, A.E.; Braughton, K.R.; Jiang, X.; DeLeo, F.R.; Chambers, H.F.; Lu, Y.; Otto, M. Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5883–5888. [CrossRef] [PubMed]

113. Chatterjee, S.S.; Joo, H.S.; Duong, A.C.; Dieringer, T.D.; Tan, V.Y.; Song, Y.; Fischer, E.R.; Cheung, G.Y.; Li, M.; Otto, M. Essential *Staphylococcus aureus* toxin export system. *Nat. Med.* **2013**, *19*, 364–367. [CrossRef] [PubMed]

114. Surewaard, B.G.; de Haas, C.J.; Vervoort, F.; Rigby, K.M.; DeLeo, F.R.; Otto, M.; van Strijp, J.A.; Nijland, R. Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell. Microbiol.* **2013**, *15*, 1427–1437. [CrossRef] [PubMed]

115. Dumont, A.L.; Nygaard, T.K.; Watkins, R.L.; Smith, A.; Kozhaya, L.; Kreiswirth, B.N.; Shopsin, B.; Unutmaz, D.; Voyich, J.M.; Torres, V.J. Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. *Mol. Microbiol.* **2011**, *79*, 814–825. [CrossRef] [PubMed]

116. Ventura, C.L.; Malachowa, N.; Hammer, C.H.; Nardone, G.A.; Robinson, M.A.; Kobayashi, S.D.; DeLeo, F.R. Identification of a novel *Staphylococcus aureus* two-component leukotoxin using cell surface proteomics. *PLoS ONE* **2010**, *5*, e11634. [CrossRef] [PubMed]

117. Panton, P.N.; Valentine, F.C.O. Staphylococcal toxin. *Lancet* **1932**, *219*, 506–508. [CrossRef]

118. Gravet, A.; Colin, D.A.; Keller, D.; Girardot, R.; Monteil, H.; Prevost, G. Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. *FEBS Lett.* **1998**, *436*, 202–208. [CrossRef]

119. Morinaga, N.; Kaihou, Y.; Noda, M. Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. *Microbiol. Immunol.* **2003**, *47*, 81–90. [CrossRef] [PubMed]

120. Nilsson, I.M.; Hartford, O.; Foster, T.; Tarkowski, A. Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. *Inf. Immun.* **1999**, *67*, 1045–1049.

121. Malachowa, N.; Whitney, A.R.; Kobayashi, S.D.; Sturdevant, D.E.; Kennedy, A.D.; Braughton, K.R.; Shabb, D.W.; Diep, B.A.; Chambers, H.F.; Otto, M.; et al. Global changes in *Staphylococcus aureus* gene expression in human blood. *PLoS ONE* **2011**, *6*, e18617. [CrossRef] [PubMed]

122. Sotto, A.; Lina, G.; Richard, J.L.; Combescure, C.; Bourg, G.; Vidal, L.; Jourdan, N.; Etienne, J.; Lavigne, J.P. Virulence potential of *Staphylococcus aureus* strains isolated from diabetic foot ulcers. *Diabetes Care* **2008**, *31*, 2318–2324. [CrossRef] [PubMed]

123. Boyle-Vavra, S.; Daum, R.S. Community-acquired methicillin-resistant *Staphylococcus aureus*: The role of Panton-Valentine leukocidin. *Lab. Investig.* **2007**, *87*, 3–9. [CrossRef] [PubMed]

124. Kaneko, J.; Kimura, T.; Kawakami, Y.; Tomita, T.; Kamio, Y. Panton-valentine leukocidin genes in a phage-like particle isolated from mitomycin C-treated *Staphylococcus aureus* V8 (ATCC 49775). *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1960–1962. [CrossRef] [PubMed]

125. Narita, S.; Kaneko, J.; Chiba, J.; Piemont, Y.; Jarraud, S.; Etienne, J.; Kamio, Y. Phage conversion of Panton-Valentine leukocidin in *Staphylococcus aureus*: Molecular analysis of a PVL-converting phage, phiSLT. *Gene* **2001**, *268*, 195–206. [CrossRef]

126. Dupieux, C.; Blondé, R.; Bouchiat, C.; Meugnier, H.; Bes, M.; Laurent, S.; Vandenesch, F.; Laurent, F.; Tristan, A. Community-acquired infections due to *Staphylococcus argenteus* lineage isolates harbouring the Panton-Valentine leucocidin, France, 2014. *Euro Surveill.* **2015**, *20*, 21154. [CrossRef] [PubMed]

127. Shallcross, L.J.; Fragaszy, E.; Johnson, A.M.; Hayward, A.C. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: A systematic review and meta-analysis. *Lancet Infect. Dis.* **2013**, *13*, 43–54. [CrossRef]

128. Gillet, Y.; Issartel, B.; Vanhems, P.; Fournet, J.C.; Lina, G.; Bes, M.; Vandenesch, F.; Piemont, Y.; Brousse, N.; Floret, D.; et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* **2002**, *359*, 753–759. [CrossRef]

129. Vandenesch, F.; Naimi, T.; Enright, M.C.; Lina, G.; Nimmo, G.R.; Heffernan, H.; Liassine, N.; Bes, M.; Greenland, T.; Reverdy, M.E.; et al. Community- acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: Worldwide emergence. *Emerg. Infect. Dis.* **2003**, *9*, 978–984. [CrossRef] [PubMed]

130. Shukla, S.K.; Karow, M.E.; Brady, J.M.; Stemper, M.E.; Kislow, J.; Moore, N.; Wroblewski, K.; Chyou, P.H.; Warshauer, D.M.; Reed, K.D.; et al. Virulence genes and genotypic associations in nasal carriage, community-associated methicillin- susceptible and methicillin-resistant USA400 *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* **2010**, *48*, 3582–3592. [CrossRef] [PubMed]

131. Kiliç, A.; Doğan, E.; Kaya, S.; Baysallar, M. Investigation of the presence of *mecC* and Panton-Valentine leukocidin genes in *Staphylococcus aureus* strains isolated from clinical specimens during seven years period. *Mikrobiyol. Bulteni* **2015**, *49*, 594–599. [CrossRef]

132. Santosaningsih, D.; Santoso, S.; Budayanti, N.S.; Suata, K.; Lestari, E.S.; Wahjono, H.; Djamal, A.; Kuntaman, K.; van Belkum, A.; Laurens, M.; et al. Characterisation of clinical *Staphylococcus aureus* isolates harbouring *mecA* or Panton-Valentine leukocidin genes from four tertiary care hospitals in Indonesia. *Trop. Med. Int. Health* **2016**, *21*, 610–618. [CrossRef] [PubMed]

133. Motamedi, H.; Rahmat Abadi, S.S.; Moosavian, S.M.; Torabi, M. The association of Panton-Valentine leukocidin and *mecA* genes in Methicillin-Resistant *Staphylococcus aureus* isolates from patients referred to Educational Hospitals in Ahvaz, Iran. *Jundishapur J. Microbiol.* **2015**, *8*, e22021. [CrossRef] [PubMed]

134. Yu, F.; Liu, Y.; Lv, J.; Qi, X.; Lu, C.; Ding, Y.; Li, D.; Liu, H.; Wang, L. Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections. *Braz. J. Infect. Dis.* **2015**, *19*, 614–622. [CrossRef] [PubMed]

135. Conceição, T.; Coelho, C.; de Lencastre, H.; Aires-de-Sousa, M. Frequent occurrence of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) strains in two African countries. *J. Antimicrob. Chemother.* **2015**, *70*, 3200–3204. [PubMed]

136. Chen, B.; Dai, X.; He, B.; Pan, K.; Li, H.; Liu, X.; Bao, Y.; Lao, W.; Wu, X.; Yao, Y.; Huang, S. Differences in *Staphylococcus aureus* nasal carriage and molecular characteristics among community residents and healthcare workers at Sun Yat-Sen University, Guangzhou, Southern China. *BMC Infect. Dis.* **2015**, *15*, 303. [CrossRef] [PubMed]

137. Morgan, M.S. Diagnosis and treatment of Panton-Valentine leukocidin (PVL)-associated staphylococcal pneumonia. *Int. J. Antimicrob. Agents* **2007**, *30*, 289–296. [CrossRef] [PubMed]

138. Rossney, A.S.; Shore, A.C.; Morgan, P.M.; Fitzgibbon, M.M.; O'Connell, B.; Coleman, D.C. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. *J. Clin. Microbiol.* **2007**, *45*, 2554–2563. [CrossRef] [PubMed]

139. Abdulgader, S.M.; Shittu, A.O.; Nicol, M.P.; Kaba, M. Molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* in Africa: A systematic review. *Front. Microbiol.* **2015**, *6*, 348. [CrossRef] [PubMed]

140. Bazzi, A.M.; Rabaan, A.A.; Fawarah, M.M.; Al-Tawfiq, J.A. Prevalence of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* infections in a Saudi Arabian hospital. *J. Infect. Public Health* **2015**, *8*, 364–368. [CrossRef] [PubMed]

141. Ohadian Moghadam, S.; Pourmand, M.R.; Mahmoudin, M.; Sadighian, H. Molecular characterization of methicillin-resistant *Staphylococcus aureus*: Characterization of major clones and emergence of epidemic clones of sequence type (ST) 36 and ST 121 in Tehran, Iran. *FEMS Microbiol. Lett.* **2015**, *362*, fnv043. [CrossRef] [PubMed]

142. Liu, X.; Liang, J.; Jiang, Y.; Wang, B.; Yuan, H.; Zhang, L.; Zhou, Y.; Xu, H.; Zhou, W. Molecular characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains isolated from outpatients with skin and soft tissue infections in Wuhan, China. *Pathog. Dis.* **2016**, *74*, ftw026. [CrossRef] [PubMed]

143. David, M.Z.; Acree, M.E.; Sieth, J.J.; Boxrud, D.J.; Dobbins, G.; Lynfield, R.; Boyle-Vavra, S.; Daum, R.S. Pediatric *Staphylococcus aureus* isolate genotypes and infections from the dawn of the Community-Associated Methicillin-Resistant *S. aureus* epidemic era in Chicago, 1994 to 1997. *J. Clin. Microbiol.* **2015**, *53*, 2486–2491. [CrossRef] [PubMed]

144. Post, V.; Wahl, P.; Uçkay, I.; Ochsner, P.; Zimmerli, W.; Corvec, S.; Loiez, C.; Richards, R.G.; Moriarty, F. Phenotypic and genotypic characterisation of *Staphylococcus aureus* causing musculoskeletal infections. *Int. J. Med. Microbiol.* **2014**, *304*, 565–576. [CrossRef] [PubMed]

145. Dunyach-Remy, C.; Courtais-Coulon, C.; DeMattei, C.; Jourdan, N.; Schuldiner, S.; Sultan, A.; Carrière, C.; Alonso, S.; Sotto, A.; Lavigne, J.P. Link between nasal carriage of *Staphylococcus aureus* and infected diabetic foot ulcers. Unpublished work, 2016.

146. Stappers, M.H.T.; Hagen, F.; Reimnitz, P.; Mouton, J.W.; Meis, J.F.; Gyssens, I.C. Direct molecular versus culture-based assessment of Gram-positive cocci in biopsies of patients with major abscesses and diabetic foot infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **2015**, *34*, 1885–1892. [CrossRef] [PubMed]

147. Tristan, A.; Bes, M.; Meugnier, H.; Lina, G.; Bozdogan, B.; Courvalin, P.; Reverdy, M.E.; Enright, M.C.; Vandenesch, F.; Etienne, J. Global distribution of Panton-Valentine leukocidin positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg. Infect. Dis.* **2007**, *13*, 594–600. [CrossRef] [PubMed]

148. Antri, K.; Rouzic, N.; Dauwalder, O.; Boubekri, I.; Bes, M.; Lina, G.; Vandenesch, F.; Tazir, M.; Ramdani-Bouguessa, N.; Etienne, J. High prevalence of methicillin-resistant *Staphylococcus aureus* clone ST80-IV in hospital and community settings in Algiers. *Clin. Microbiol. Infect.* **2011**, *17*, 526–532. [CrossRef] [PubMed]

149. Ben Nejma, M.; Mastouri, M.; Bel Hadj Jrad, B.; Nour, M. Characterization of ST80 Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* clone in Tunisia. *Diagn. Microbiol. Infect. Dis.* **2008**, *77*, 20–24. [CrossRef] [PubMed]

150. Lamy, B.; Laurent, F.; Gallon, O.; Doucet-Populaire, F.; Etienne, J.; Decousser, J.W. Antibacterial resistance, genes encoding toxins and genetic background among *Staphylococcus aureus* isolated from community-acquired skin and soft tissue infections in France: A national prospective survey. *Eur. J. Clin. Microbiol. Infect. Dis.* **2012**, *31*, 1279–1284. [CrossRef] [PubMed]

151. Durupt, F.; Mayor, L.; Bes, M.; Reverdy, M.E.; Vandenesch, F.; Thomas, L.; Etienne, J. Prevalence of *Staphylococcus aureus* toxins and nasal carriage in furuncles and impetigo. *Br. J. Dermatol.* **2007**, *157*, 1161–1167. [CrossRef] [PubMed]

152. Issartel, B.; Tristan, A.; Lechevallier, S.; Bruyère, F.; Lina, G.; Garin, B.; Lacassin, F.; Bes, M.; Vandenesch, F.; Etienne, J. Frequent carriage of Panton-Valentine leucocidin genes by *Staphylococcus aureus* isolates from surgically drained abscesses. *J. Clin. Microbiol.* **2005**, *43*, 3203–3207. [CrossRef] [PubMed]

153. Puzniak, L.A.; Quintana, A.; Wible, M.; Babinchak, T.; McGovern, P.C. Methicillin-resistant *Staphylococcus aureus* infection epidemiology and clinical response from tigecycline soft tissue infection trials. *Diagn. Microbiol. Infect. Dis.* **2014**, *79*, 261–265.

154. Senneville, E.; Brière, M.; Neut, C.; Messad, N.; Lina, G.; Richard, J.L.; Sotto, A.; Lavigne, J.P.; French Study Group on the Diabetic Foot. First report of the predominance of clonal complex 398 *Staphylococcus aureus* strains in osteomyelitis complicating diabetic foot ulcers: A national French study. *Clin. Microbiol. Infect.* **2014**, *20*, O274–O277. [CrossRef] [PubMed]

155. Alonzo, F., 3rd; Benson, M.A.; Chen, J.; Novick, R.P.; Shopsin, B.; Torres, V.J. *Staphylococcus aureus* leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. *Mol. Microbiol.* **2012**, *83*, 423–435. [CrossRef] [PubMed]

156. Feng, S.H.; Chu, Y.J.; Wang, P.H.; Jun, X.; Min, D.; Li, X.M. Risk factors and gene type for infections of MRSA in diabetic foot patients in Tianjin, China. *Int. J. Low. Extrem. Wounds* **2013**, *12*, 106–112. [CrossRef] [PubMed]

157. Nhan, T.X.; Leclercq, R.; Cattoir, V. Prevalence of toxin genes in consecutive clinical isolates of *Staphylococcus aureus* and clinical impact. *Eur. J. Clin. Microbiol. Infect. Dis.* **2011**, *30*, 719–725. [CrossRef] [PubMed]

158. Becker, K.; Friedrich, A.W.; Lubritz, G.; Weilert, M.; Peters, G.; von Eiff, C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J. Clin. Microbiol.* **2003**, *41*, 1434–1439. [CrossRef] [PubMed]

159. Sila, J.; Sauer, P.; Kolar, M. Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, Panton-Valentine leukocidin and Tsst-1 between methicillin-resistant and methicillin-susceptible isolates of *Staphylococcus aureus* at the University Hospital in Olomouc. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* **2009**, *153*, 215–218. [CrossRef] [PubMed]

160. Spaulding, A.R.; Salgado-Pabon, W.; Kohler, P.L.; Horswill, A.R.; Leung, D.Y.; Schlievert, P.M. Staphylococcal and streptococcal superantigen exotoxins. *Clin. Microbiol. Rev.* **2013**, *26*, 422–447. [CrossRef] [PubMed]

161. Vu, B.G.; Stach, C.S.; Salgado-Pabon, W.; Diekema, D.J.; Gardner, S.E.; Schlievert, P.M. Superantigens of *Staphylococcus aureus* from patients with diabetic foot ulcers. *J. Infect. Dis.* **2014**, *210*, 1920–1927. [CrossRef] [PubMed]

162. Lina, G.; Bohach, G.A.; Nair, S.P.; Hiramatsu, K.; Jouvin-Marche, E.; Mariuzza, R. Standard nomenclature for the superantigens expressed by *Staphylococcus*. *J. Infect. Dis.* **2004**, *189*, 2334–2336. [CrossRef] [PubMed]

163. Diegelmann, R.F.; Evans, M.C. Wound healing: An overview of acute, fibrotic and delayed healing. *Front. Biosci.* **2004**, *9*, 283–289. [CrossRef] [PubMed]

164. Dinges, M.M.; Orwin, P.M.; Schlievert, P.M. Exotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev.* **2000**, *13*, 16–34. [CrossRef] [PubMed]

165. Lee, H.W.; Kim, S.M.; Kim, J.M.; Oh, B.M.; Kim, J.Y.; Jung, H.J.; Lim, H.J.; Kim, B.S.; Lee, W.J.; Lee, S.J.; et al. Potential immunoinflammatory role of staphylococcal enterotoxin A in atopic dermatitis: Immunohistopathological analysis and in vitro assay. *Ann. Dermatol.* **2013**, *25*, 173–180. [CrossRef] [PubMed]

166. Wilson, G.J.; Seo, K.S.; Cartwright, R.A.; Connelley, T.; Chuang-Smith, O.N.; Merriman, J.A.; Guinane, C.M.; Park, J.Y.; Bohach, G.A.; Schlievert, P.M.; et al. A novel core genome-encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia. *PLoS Pathog.* **2011**, *7*, e1002271. [CrossRef] [PubMed]

167. Boquet, P.; Lemichez, E. Bacterial virulence factors targeting Rho GTPases: Parasitism or symbiosis? *Trends Cell Biol.* **2003**, *13*, 238–246. [CrossRef]

168. Boyer, L.; Doye, A.; Rolando, M.; Flatau, G.; Munro, P.; Gounon, P.; Clément, R.; Pulcini, C.; Popoff, M.R.; Mettouchi, A.; et al. Induction of transient macroapertures in endothelial cells through RhoA inhibition by *Staphylococcus aureus* factors. *J. Cell Biol.* **2006**, *173*, 809–819. [CrossRef] [PubMed]

169. Lemichez, E.; Lecuit, M.; Nassif, X.; Bourdoulous, S. Breaking the wall: Targeting of the endothelium by pathogenic bacteria. *Nat. Rev. Microbiol.* **2010**, *8*, 93–104. [CrossRef] [PubMed]

170. Rolando, M.; Munro, P.; Stefani, C.; Auberger, P.; Flatau, G.; Lemichez, E. Injection of *Staphylococcus aureus* EDIN by the *Bacillus anthracis* protective antigen machinery induces vascular permeability. *Infect. Immun.* **2009**, *77*, 3596–3601. [CrossRef] [PubMed]

171. Munro, P.; Benchetrit, M.; Nahori, M.A.; Stefani, C.; Clément, R.; Michiels, J.F.; Landraud, L.; Dussurget, O.; Lemichez, E. The *Staphylococcus aureus* epidermal cell differentiation inhibitor toxin promotes formation of infection foci in a mouse model of bacteremia. *Infect. Immun.* **2010**, *78*, 3404–3411. [CrossRef] [PubMed]

172. Inoue, S.; Sugai, M.; Murooka, Y.; Paik, S.Y.; Hong, Y.M.; Ohgai, H.; Suginaka, H. Molecular cloning and sequencing of the epidermal cell differentiation inhibitor gene from *Staphylococcus aureus*. *Biochem. Biophys. Res. Commun.* **1991**, *174*, 459–464. [CrossRef]

173. Wilde, C.; Aktories, K. The Rho-ADP-ribosylating C3 exoenzyme from *Clostridium botulinum* and related C3-like transferases. *Toxicon* **2001**, *39*, 1647–1660. [CrossRef]

174. Franke, G.C.; Böckenholt, A.; Sugai, M.; Rohde, H.; Aepfelbacher, M. Epidemiology, variable genetic organization and regulation of the EDIN-B toxin in *Staphylococcus aureus* from bacteraemic patients. *Microbiology* **2010**, *156*, 860–872. [CrossRef] [PubMed]

175. Yamaguchi, T.; Hayashi, T.; Takami, H.; Ohnishi, M.; Murata, T.; Nakayama, K.; Asakawa, K.; Ohara, M.; Komatsuzawa, H.; Sugai, M. Complete nucleotide sequence of a *Staphylococcus aureus* exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. *Infect. Immun.* **2001**, *69*, 7760–7771. [CrossRef] [PubMed]

176. Czech, A.; Yamaguchi, T.; Bader, L.; Linder, S.; Kaminski, K.; Sugai, M.; Aepfelbacher, M. Prevalence of Rho-inactivating epidermal cell differentiation inhibitor toxins in clinical *Staphylococcus aureus* isolates. *J. Infect. Dis.* **2001**, *184*, 785–788. [CrossRef] [PubMed]

177. Munro, P.; Clément, R.; Lavigne, J.P.; Pulcini, C.; Lemichez, E.; Landraud, L. High prevalence of *edin-C* encoding RhoA-targeting toxin in clinical isolates of *Staphylococcus aureus*. *Eur. J. Clin. Microbiol. Infect. Dis.* **2011**, *30*, 965–972. [CrossRef] [PubMed]