Scenery of *Staphylococcus* implant infections in orthopedics

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Infection is still the major complication of orthopedic implants and projections based on the actual trend indicate that total hip and knee arthroplasties and their consequent infection burden are destined to greatly increase. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the leading etiologic agents of orthopedic implant infection. Here we report on epidemiology of implant-related *Staphylococcus* infections in orthopedics, also referring to our experience, and focus on the crucial role of bacterial adhesins and on their ability to direct the pathogenesis process. Bacteria initiate implant infection by adhering to biomaterials. In the early steps of infection, adhesins mediate the specific interaction between microbial cells and the extracellular matrix proteins filming biomaterial surface. Then adhesin-mediated anchorage allows bacteria to cling to the biomaterial surface and to produce a biofilm that favors their ability to resist antibiotics. With the aim to prevent implant-related infections, anti-infective and infection-resistant biomaterials are being developed. The research for novel therapeutic strategies is incited by the emergence of antibiotic-resistant bacteria. Vaccines against the adhesins or antisense molecules against virulence genes can open a future in combating implant infections.

The scenery of orthopedic implants: projections of the number of primary & revision arthroplasties

In orthopedics, implant procedures to restore the function of affected joints, fractured bone segments and impaired limbs are progressively boosting. The expansion of joint replacement surgery and the need to follow the outcome and costs of the arthroplasties booming have promoted the foundation of many registries at the regional, national and international level. The registries provide important information on the long-term effectiveness and quality of implants. The structure, functioning and content of many arthroplasty registers are examined and evaluated in a recent comprehensive review [1].

The number of primary and revision arthroplasties is increasing constantly worldwide [2]. Projections of the number of primary and revision total hip and knee arthroplasties that will be performed in the USA until 2030 have been formulated and the percent increases in respect to 2005 have been estimated [2]. By 2030, the demand for primary total hip arthroplasties is estimated to reach 572,000, with an increase of 174%. The demand for primary total knee arthroplasties will reach 3.48 million, an increase of 673% (FIGURE 1 & BOX 1).

The National Joint Registry (NJR) for England and Wales, 7th Annual Report, 2010, has now collected more than 905,000 procedures registered, making the NJR by far the largest registry of its kind in the world. In 2009, there were 72,432 hip replacement procedures recorded on the NJR. Of these, 65,229 were primary procedures and 7203 were revision surgeries, representing a revision 'burden' of 10%. Indications for revision were aseptic loosening (56%) and infection (3%).

The number of knee replacement procedures recorded on the NJR during 2009 was 77,545. There were 4565 revision procedures, representing 5.9% of the total number of knee replacement procedures in 2009 (FIGURE 2).

Infection burden for hip & knee arthroplasties

For a long time experimental evidence has been gathered that the presence of a foreign body reduces the minimal inoculum of *S. aureus* that is required to cause infection by a factor of more than 100,000 [3,4]. Experimental observations with animal models show that in the presence of a foreign body, infection may occur despite the use of perioperative antimicrobial prophylaxis, since fewer than 100 CFU of microorganisms

Keywords

■ adhesins ■ antibiotic-loaded biomaterials ■ biofilm ■ DNA vaccines ■ implant infections ■ infection-resistant materials ■ MSCRAMMs ■ orthopedic implants ■ peptide-nucleic-acids ■ *Staphylococcus aureus* ■ *Staphylococcus epidermidis* ■ vaccines

Figure 1. Projections of worldwide increase of arthroplasties from 2005 to 2030.

can induce infection [5]. The conclusions drawn from experimental models also hold in human pathology. Murdoch *et al.* observed that during *S. aureus* bacteremia, an implant-associated infection developed in 15 out of 44 patients with prosthetic joints. Thus, infection can occur not only during surgery but during the entire lifetime of the implant [6]. The study of Murdoch, showing a high incidence of hematogenous seeding for *S. aureus*, was occasionally criticized because it was the only one of its kind. Afterwards two further studies have confirmed Murdoch's data. If fact, Lalani *et al.* reported that patients with *S. aureus* bacteremia and orthopedic implant had higher relapse of infection, compared with bacteremic patients without implant [7]. Sendi *et al.* evaluated the incidence of hematogenous periprosthetic joint infections in patients with *S. aureus* bacteremia and concluded that the rate of periprosthetic joint infections after *S. aureus* bacteremia is high, ranging from 30 to 40%, and clearly higher than rates reported for bacteremia by other pathogens [8].

Infection following total hip and knee arthroplasties remains a major complication in joint

reconstruction, resulting in significant morbidity to the patient and increased hospital costs. To quote the words of Sculco written back in 1993 "Infection in total joint replacement is a devastating and life-threatening complication for the patient. It can also be an economic disaster for hospitals that treat large numbers of these patients" [9]. Since then, things may have changed, but implant infection still remains a health and economic problem. The current and historical incidence of periprosthetic infection associated with hip and knee arthroplasty in the USA, as well as the corresponding hospitalization charges and the lengths of stay have been calculated using the Nationwide Inpatient Sample. According to Kurtz *et al.* [2], to Katz *et al.* [10] and to Mahomed *et al.* [11] the short-term risk of periprosthetic infection ranges between 0.2 and 0.4% for primary total hip arthroplasty and total knee arthroplasty, respectively.

Applying these estimates to the expected increase of arthroplasties in the USA and in the UK, the infections for hip and knee arthroplasties may reach worrying numbers according to Jämsen *et al.* [12].

Currently the infection rates are approximately 1% in primary knee replacements and 0.3–0.6% in hip replacements [13–15]. However, even prospective surveillance programs may underestimate the infection rates; thus, the true incidence is probably higher [16,17].

Currently, periprosthetic infection is most frequently diagnosed by the isolation of one or more microorganisms from periprosthetic tissue or fluid with the use of conventional microbiologic culture techniques, and the results of microbiologic culture are usually considered the standard with which other diagnostic tests are compared.

The current classification of American Association of Orthopedic Surgeons divides prosthetic infections into the following four types [18]:

- Type 1 (positive intraoperative culture): two intraoperative cultures turning out positive;

Box 1. Future demand for arthroplasties.

- Estimated demand increase of primary total hip in the USA until 2030: 174%, reaching 572,000
- Estimated demand increase of primary total knee in the USA until 2030: 673%, reaching 3.48 million
- Estimated demand of revision of total hip in the USA will double by the year 2026
- Estimated demand of revision of total knee in the USA will double by the year 2015

Incidence of infections in joint replacement surgery

- Primary knee replacements: 1.3% [12,17]
- Primary hip replacements: 1.6% [13–15,17]

- Type 2 (early postoperative infection): infection occurring within the first month after surgery;
- Type 3 (acute hematogenous infection): hematogenous seeding of site of previously well-functioning prosthesis;
- Type 4 (late chronic infection): chronic indolent clinical course; infection present for more than 1 month.

In a slightly different classification by Zimmerli *et al.* [19], infections associated with prosthetic joints are divided into the following categories:

- Early (those that develop less than 3 months after surgery);
- Delayed (3–24 months after surgery), or;
- Late (more than 24 months after surgery).

The most common bacteria isolated from infected joints are coagulase-positive or -negative *Staphylococcus* [20,21]. The infecting organisms are either introduced during implantation of a prosthesis or derived from a temporary bacteremia. They then adhere to biomaterials and grow to form a biofilm. The mode of growth in biofilm offers enhanced protection against natural host defenses and antibiotic therapies [22].

Epidemiology of implant-related *Staphylococcus* infections in orthopedics in our experience

In the past we have conducted a large study on a collection of 1027 isolates, obtained in the period between 2000 and 2003 from 699 orthopedic patients bearing infections [21]. The results compared the etiology of infections associated

with medical devices (MDs) to those developed in the absence of implant materials (no MDs). MDs included infections associated to knee and hip prostheses, external and internal fractured bone fixation systems, materials for tendon and ligament reconstructions and other orthopedic implant materials. *Staphylococcal* species were found to be the most prevalent etiological agents of orthopedic infections, representing 75.3% of all strains, 78.1% of the isolates obtained from MDs and just 68.3% of those from the no MD group. Among the species belonging to the *Staphylococcus* genus, *S. aureus* generally exhibited the highest prevalence (35.5% overall prevalence, 33.8% in MDs and 40.3% in the no MD group), closely approached by *Staphylococcus epidermidis* only in the presence of a foreign body. No single bacterial species, except for *S. aureus* and *S. epidermidis*, exceeded a frequency of 7%, giving emphasis to the critical importance of these two species in the epidemiology of orthopedic infections [21].

Recently we have analyzed an analog collection of isolates from 242 orthopedic patients referring to the period between 2007 and 2011. The results derived from the new study matched rather well with, and confirmed those of, the old epidemiological investigation. Again the prevalence of staphylococci in the entire collection was approximately 75%, slightly higher in the case of MDs (82.3%) and lower for no MDs (65.4%). TABLE 1 reports in detail each etiological agent identified and the respective frequency based on the category of infection. *S. aureus* again represents approximately 35% of all the isolates. The coagulase-negative species are singularly reported in order to show the variety of minor staphylococcal species isolated from

Figure 2. Hip and knee arthroplasties recorded by the National Joint Registry for England and Wales (cases of 2009).

Table 1. Etiology of orthopedic infections (strains sampled in the period 2007–2011).

Microorganism	All		MDs		No MDs	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<i>Staphylococcus aureus</i>	95	35.1	52	31.7	43	40.2
<i>Staphylococcus epidermidis</i>	81	29.9	64	39.0	17	15.9
<i>Pseudomonas aeruginosa</i>	25	9.2	10	6.1	15	14.0
<i>Enterococcus faecalis</i>	11	4.1	4	2.4	7	6.5
<i>Staphylococcus haemolyticus</i>	9	3.3	3	1.8	6	5.6
<i>Escherichia coli</i>	8	3.0	4	2.4	4	3.7
<i>Staphylococcus warneri</i>	8	3.0	8	4.9	0	0.0
<i>Staphylococcus hominis</i>	5	1.8	4	2.4	1	0.9
<i>Streptococcus agalactiae</i>	4	1.5	1	0.6	3	2.8
<i>Proteus mirabilis</i>	4	1.5	2	1.2	2	1.9
<i>Staphylococcus lugdunensis</i>	3	1.1	2	1.2	1	0.9
<i>Acinetobacter baumannii</i>	3	1.1	1	0.6	2	1.9
<i>Enterobacter cloacae</i>	3	1.1	2	1.2	1	0.9
<i>Staphylococcus capitis</i>	3	1.1	1	0.6	2	1.9
<i>Enterococcus faecium</i>	2	0.7	1	0.6	1	0.9
<i>Morganella morganii</i>	2	0.7	2	1.2	0	0.0
<i>Staphylococcus cohnii</i>	1	0.4	1	0.6	0	0.0
<i>Corynebacterium striatum</i>	1	0.4	0	0.0	1	0.9
<i>Serratia</i> spp.	3	1.1	2 [†]	1.2	1 [*]	0.9
Total:	271		164		107	

[†]*Serratia marcescens* and a still unresolved species of *Serratia*.

^{*}*Serratia liquefaciens*.

MD: Medical devices.

orthopedic infections, representing new pathogens in implant infections [23]. We observed that the prevalence of *S. epidermidis* has increased from 31.5% in the old collection to 39.0% in the new collection (χ^2 , *p* value = 0.06), revealing that this coagulase-negative *Staphylococcus* is progressively prevailing on *S. aureus*. The only statistically significant difference emerging from the analysis of the first five most prevalent species concerned *Pseudomonas aeruginosa*, which increased among the no MDs group from the earlier observed 6.8% to the current 14.0% (χ^2 , *p* value = 0.026), remaining actually unchanged in the case of MDs. FIGURE 3 shows that *S. aureus* has been found to be the main pathogen among infections in the no MD group and infections associated with internal and external fixation systems, while *S. epidermidis* was the most prevalent bacterial species among infections associated with knee and hip arthroplasties.

Overall, our findings enlighten and further substantiate the abundant existing literature on the role of *S. aureus* in postsurgical orthopedic infections. At the same time they indicate that the role of *S. aureus* is different in the presence of MDs as a function of the category of implant and the conditions of application.

The complex role of microbial surface components recognizing adhesive matrix molecules adhesins in the early steps of infection

The particular versatility of *S. aureus* to originate distinct disease conditions with diverse clinical manifestations parallels the highly clonal and adaptable nature of this pathogen [24]. The identification of specific traits of virulence expressed by *S. aureus* strain types has directed most molecular epidemiology studies over the last decade. However, the particularly rich array of putative virulence factors encoded in the *S. aureus* genome has impeded many of the attempts to reveal a dominant virulence factor. The long-lasting controversy regarding the disputed role of the Panton-Valentine leukocidin in the development of skin infections caused by community-acquired methicillin-resistant *S. aureus* is an explicative example. After years of investigations and disputes, the real role played by Panton-Valentine leukocidin still remains unresolved [25].

In *S. aureus*, the so called ‘virulon’ consists of approximately 50 accessory genes [26], encoding for factors either secreted or expressed on the bacterial surface, all having a function in infection pathogenesis. These factors include toxins and a

variety of other molecules mediating anti-inflammatory interactions, immune evasion, bacterial adhesion and colonization of host tissues, bio-film formation and host-cell invasion. In such context, adhesins represent an important group of virulence factors mediating surface adhesive interactions between microbial cells and host cells and extracellular matrix (ECM). They play a primary role, particularly in the early phases of infection, fulfilling tissue tropism, surface adhesion and colonization. However, specific adhesins have been found to act even as invasins and, furthermore, intervene in the process of bacterial internalization into host cells. Surface adhesion is a critical step in the pathogenesis of implant-related infections and represents the initial start of a process opening the way to the colonization of biomaterial surfaces. For this reason, in the early 1990s the influence of adhesins on the adhesion and colonization of implant surfaces became a field of intense study [27–30]. Passive bacterial adsorption spontaneously occurs on material surfaces, but active stable anchorage of the microbial cells is established by adhesins (FIGURE 4), which bind to host proteins adsorbed on the implant surface following exposure to physiologic fluids.

S. aureus adhesins group includes the cell wall-anchored microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [31,32], as well as the recently described secretable expanded repertoire adhesive molecules (SERAMs), ionically rather than covalently associated to the bacterial cell wall [33]. MSCRAMMs, unlike SERAMs, are receptorial proteins anchored to the bacterial cell wall through a typical cell wall sorting signal Leu-Pro-X-Thr-Gly (LPXTG) motif. The *S. aureus* enzyme sortase A, located on the extracellular side of the membrane, cleaves the LPXTG motif, covalently anchoring the adhesin to the cell wall peptidoglycan [34]. Bound to the peptidoglycan and exposed on the bacterial surfaces, MSCRAMMs recognize specific ECM components. Their function is far from being totally clarified as the complex structure of MSCRAMMs often comprises multiple domains recognizing different ECM ligands or, alternatively, single domains with affinity for multiple alternative ligands. Through the interaction with ECM proteins, certain MSCRAMMs acting as invasins can facilitate the process of internalization into host cells. Other MSCRAMMs mediate bacterial cell accumulation, contributing to

Figure 3. Prevalence of the five most frequent pathogens as a function of the origin of the orthopedic infection in a collection of 272 clinical isolates obtained from 242 patients in the period between 2007 and 2011. Confirming previous data, *Staphylococcus aureus* was found to be the main pathogen among infections not associated with medical devices and infections associated to IF and EF systems. Conversely, *Staphylococcus epidermidis* was the most prevalent bacterial species among infections associated to K and H arthroprostheses. EF: External fixation; H: Hip; IF: Internal fixation; K: Knee; MD: Medical device.

Figure 4. Bacterial attachment and fixation to biomaterial surfaces by a bacterial active process. Pathogens such as *Staphylococcus aureus* possess receptorial adhesins that bind to specific host proteins eventually adsorbed on the biomaterial surface. Initial bacterial adhesion can be followed by the formation of mature biofilms. At present, a number of alternative mechanisms are known that can enable biofilm formation: **(i)** the production of a glycocalyx consisting of exopolysaccharidic-cementing substances enabling bacterial intercellular aggregation and subsequent encasement; **(ii)** the release of eDNA derived from mechanisms of altruistic suicide (or fratricidal microbial lysis in *Enterococcus faecalis*); **(iii)** finally, various protein factors are thought to be implicated in biofilm production, among them FnBPs, Aap, Bap and SpA.

biofilm formation by means of mechanisms alternative to the classical formation of that exopolysaccharide typical of most *S. aureus* strains and associated to the expression of an *ica* locus encoding for the polysaccharide intercellular adhesin (PIA). TABLE 2 reports a synoptic view of the multifaceted functions of most investigated *S. aureus* MSCRAMMs, enlightening at the same time the complexity of interactions actuated with host ECM components and the still large gap in terms of knowledge to be fulfilled. A number of SERAMs have been found to be associated with

endovascular infections. Among them, coagulase, the extracellular fibrinogen-binding protein, the ECM-binding protein and the extracellular adhesive protein have been described to be the most prominent ones [33].

Prevalence of adhesin genes in collections of *S. aureus* clinical isolates

The role played by virulence factors can be investigated through a variety of experimental models and strategies, which include: *in vitro* and *in vivo* comparative models based on wild and mutant

Table 2. Known microbial surface components recognizing adhesive matrix molecules: ligand specificities and reported functions.

Adhesin name	Acronym	Ligand(s)	Activities, mechanism and putative role in pathogenesis
Collagen-binding protein	CNA	Collagen (collagen triple-helix monomer)	Activities: reported to bind to collagen just in its monomeric form and not to fibrillar collagen [128]; adhesion to cartilage tissue [129]. The interaction between CNA/collagen type I appears important in thrombus development in whole blood under shear conditions [130] Animal models: virulence factor in endocarditis, osteomyelitis, arthritis and keratitis [131–133]. Expression: high in exponential growth phase Prevalence: high in clinical isolates
Fibronectin-binding protein A	FnBPA	Fibronectin Elastin Fibrinogen	Activities: prominent role in attachment and host tissue colonization; binding of fibronectin at the domain near the C-terminus, specifically inhibited by heparin [95,96]; platelet activation and aggregation (fibrinogen and/or fibronectin bridge with platelet integrin GPIIb/IIIa and crosslink to IgG FcγRIIIa receptor by antibodies to both A domain and repetitive domain [134]); invasin (internalization via fibronectin bridging to integrin $\alpha_5\beta_1$ of host cells) [135]; endothelial colonization and tissue invasion (via fibronectin bridging to integrin $\alpha_5\alpha_1$ of host cells) [136]; induction of proinflammatory and procoagulant endothelial responses [137]; biofilm development [138]
Fibronectin-binding protein B	FnBPB	Fibronectin Elastin	Animal models: virulence factor in endocarditis [139]
Iron-regulated surface determinants	IsdA	Transferrin, fibrinogen, fibronectin, fetuin, involucrin, haemin, loricrin and CK10	Activities: cell wall-anchored surface receptors of the Isd system are involved in heme-iron uptake [140]; protection from bactericidal activity of apolactoferrin [141]; adherence to desquamated nasal epithelial cells [142];
	IsdB	Human hemoglobin, haemin	Animal models: importance to iron acquisition and staphylococcal pathogenicity in murine models of infection. Decreased proliferation of <i>Staphylococcus aureus</i> strains inactivated for IsdA, IsdB or IsdC in heart, kidney and spleen abscess murine models [143–145]. Enhanced colonization of nares in cotton rat [146]
	IsdC	Haemin	Expression: upregulated during conditions of iron starvation
	IsdH	Haptoglobin, Haemoglobin–haptoglobin complex	
Protein A	SpA	IgG (Fc region) IgM (heavy-chain variable domain 3, VH3) von Willebrand factor gC1qR/p33 complement receptor TNFR1 receptor	Activities: following binding to IgG: impaired recognition by Fc receptors of neutrophils interfering with phagocytosis [147]; hindered activation of classical pathway of complement fixation [148]. T cell-independent B-cell proliferation directly triggered by IgM–SpA binding with consequent depletion of B-cell repertoire [149]. Bacterial adhesion to platelets and damaged vasculature through vWF-binding, and activated platelet gC1qR/p33 receptor [150]. Binding to the TNFR1 receptor for TNF- α on airway epithelium essential for the pathogenesis of pneumonia [151]. Shedding interference with TNF- α signalling on macrophages and epithelial cells [152]. Activation of type I interferon cascade [153]. Essential component in biofilm development [154].
Serine-rich adhesin for platelets	SraP (or SasA)	Unknown	Activities: binding to platelets [155] Animal models: virulence factor in infective endocarditis

Table 2. Known microbial surface components recognizing adhesive matrix molecules: ligand specificities and reported functions (cont.).

Adhesin name	Acronym	Ligand(s)	Activities, mechanism and putative role in pathogenesis
Sdr family proteins			
Clumping factor A	ClfA	Fibrinogen (C-terminal end of the γ -chain) Complement factor I	Activities: blood clumping, platelet activation and aggregation (through fibrinogen bridge with platelet integrin GPIIb/IIIa and engagement of the IgG Fc γ RIIa receptor by antibodies for the ClfA A domain [156]) and immune evasion through: inactivation of complement component C3b to iC3b [157], inhibition of leukocyte phagocytosis [158]. Greater proaggregatory activity than ClfB Animal models: virulence factor in mouse septic arthritis, rabbit and rat infective endocarditis [159–161] Expression: all stages of cell growth Prevalence: observed in nearly all clinical isolates
Clumping factor B	ClfB	Fibrinogen (flexible connector region of A α -chain) Cytokeratin 10 Cytokeratin 8	Activities: platelet aggregation (through fibrinogen bridge with platelet integrin GPIIb/IIIa and Fc receptor clustering by specific antibodies anti-ClfB or a complement-dependent mechanism) [162]; a major determinant of adherence to squamous cells in the process of colonization of anterior nares [142,163–166] Expression: exponential growth phase
Serine-aspartate dipeptide repeat proteins	SdrC	β -Neurexin 1	Activities: the presence of particular Sdr genes is correlated with putative tissue specificity. SdrC greatly enhances adherence to cultured mammalian cells expressing β -neurexin (a role in sepsis-associated polynuropathy?) [167]. SdrC and SdrD each contributed to the ability of <i>S. aureus</i> to adhere to squamous cells [140]. Possible role of SdrD in the interaction between the pathogen and the immune system. Depending on the different reports, higher prevalence of SdrD or both SdrD and SdrE in isolates from bone infections [168,169]. SdrE can induce platelet aggregation [170]
	SdrD	Unknown	Prevalence: presence of at least two genes of Sdr protein tandemly arrayed. The gene of SdrC is usually present in most isolates. In the USA, clinical isolates of all three Sdr proteins are more associated with MRSA than MSSA [168]
	SdrE	Unknown	
Bone sialoprotein-binding protein (variant form of SdrE)	Bbp	Bone sialoprotein (osteopontin)	Activities: role in osteomyelitis owing to bone tissue tropism [171]. Antibodies to Bbp are indicative of infectious osteomyelitis [172,173]

strains that are defective for a given virulence determinant; *in vitro* and *in vivo* models comparing groups of strains exhibiting or lacking such a determinant; transfer of the gene for a putative virulence trait in a nonpathogen species; molecular epidemiology studies assessing the distribution of the genes encoding for virulence factors in well-characterized collections of invasive, colonizing, contaminating or commensal strains; and molecular epidemiology studies surveying associations of well-defined virulence traits with newly confirmed emerging epidemic strain types. These different approaches offer a number of advantages and drawbacks. *In vitro* as well as *in vivo* animal models are often oversimplistic and unrepresentative of real clinical conditions involving human patients, even though experiments can be performed under appropriately selected and controlled conditions. Conversely, studies based on clinical isolates require long-term collection and storage of a vast number of isolates, their extensive molecular characterization, including strain subtyping and a full access to the data concerning the history of the patient and origin and development of the infection. Only recently, with complete sequencing of genomic DNA from different strain types, the selection of primers for PCR screening of target genes could be performed, keeping in consideration both stable and highly variable regions of adhesin genes. Thus, initial molecular epidemiology studies, blindly relying on subjectively chosen primers, often led to noncomparable results among different authors.

Our earliest studies, in line with the efforts made by other pioneers investigating the pathogenesis of implant-related infections, were aimed at elucidating the role and weight of selected MSCRAMMs and of biofilm formation, which were thought to be relevant to the virulence of staphylococcal strains in the presence of orthopedic prosthetic materials [35–39]. At the time, the plausible involvement of MSCRAMMs in the processes of tissue tropism via active bacterial adhesion to tissue-specific ECM components and on ECM proteins-coated surfaces was strongly suggestive of a primary role of these factors in determining the colonization and subsequent infection of implants. Nevertheless, despite all these mounted expectations to identify a single adhesin or virulence factor capable of discriminating saprophytic contaminants from invasive pathogenic strains, the investigations inexorably ended showing at most a slightly increased prevalence of certain virulence traits among clinical isolates and never a neat ‘present or absent’

response. In 2000, Peacock *et al.* reported that strains positive for both fibronectin-binding protein (FnBP) A and FnBPB ranged from 85.3% in endocarditis, 83.9% in septic arthritis/osteomyelitis and 75.1% in orthopedic implant-associated infections, down to 63.6% for nasal carriage isolates [40]. Therefore, even when considering the combination of important adhesins mediating the interaction with fibronectin, the difference between nasal carriage and implant associated-infections was less than 12% in frequency. In another more articulated study, the presence of different virulence determinants, including adhesins, toxins, factors implicated in biofilm formation and others, was screened among carriage and invasive isolates [41]. The authors found that seven determinants, among them the genes encoding for three MSCRAMMs (respectively for FnBPA, collagen-binding protein [CNA], and serine-aspartate dipeptide repeat proteins), were significantly more common in invasive isolates. The effect of these genes was cumulative, each independently multiplying the odds of disease. The prevalence of these genes differed from each other by a maximum of 20% in carriage and in invasive strains. However, this last work opened a new perspective, emphasizing that the virulence potential of each single strain is of multifactorial nature, implying that the recognition of an invasive strain has to rely on a more complex assessment of its virulon.

Therefore, virulence, as a survival strategy adopted by a given strain, is not simply a property associated to a single determinant, but should rather be regarded as the result of a combination of traits, including adhesins, invasins and toxins, whose expression is successfully ruled by appropriate polymorphisms of regulatory genes. In this regard, the accessory gene regulator (*agr*) locus, associated to the quorum sensing system in *S. aureus*, is known to orchestrate the expression of many toxin genes and adhesion molecules and could therefore play a pivotal role (see Biofilm section).

In line with these considerations, our recent findings point to distinct patterns of virulence genes, which underlie distinct evolutive strategies associated with the polymorphisms of the *agr* locus in *S. aureus* causing orthopedic implant infections [42]. Isolates presenting different *agr* polymorphisms appear to base their survival strategies on diverse alternative adhesins or sets of adhesins. From the analysis of 200 *S. aureus* isolates derived from implant-related infections, we observed that the isolates belonging to the *agr* type III was found to be associated with the

presence of bone sialoprotein-binding protein (Bbp; generally absent in isolates of our collection with *agr* type I and II groups) and with the copresence of Bbp and CNA. In a further study we compared the frequency of adhesin genes in epidemic and sporadic isolates derived from implant-related orthopedic infections. In the collection we observed that the most prevalent ribogroup was characterized by the tandem arrangement of two adhesin genes, respectively encoding for CNA and Bbp [43]. There is some evidence that in veterinary microbiology the *bbp* gene, encoding for Bbp, is present only in typical high virulent *S. aureus* strains [44]. It was rather intriguing for us to observe that the most epidemic and successful strain type among biomaterial-associated orthopedic infections is largely represented by isolates endowed with both Bbp and CNA, two adhesins whose respective ligands represent by far the main body of ECM protein components.

The failure of many attempts to ascertain a clear correlation between single molecular determinants and clinical outcomes is lately pushing to a more comprehensive approach, nonlimited to well-defined virulon members, but considering the entire genome, including coding sequences as well as noncoding sequences [45]. Probably, for highly clonal pathogens causing a broad range of diseases, as in the case of *S. aureus*, the complexity is such to implicate different models and infective strategies, each adequate or sufficient for the different conditions of the patients, either healthy and perfectly immunocompetent or with various grades of breached immune defenses. The challenging objective of future genomic and postgenomic eras will be at least to uncover and define the essential and most critical virulence mechanisms among prevailing and most worrying strain types. A better understanding of the role of adhesins and toxins in most prevalent epidemic strains and their added value in terms of acquired virulence will be the most rational approach to elaborate new anti-infective strategies with the largest impact and alternative to antibiotics. In this context, implant infections are particularly relevant because of their impact on the human population in terms of numbers and critical consequences, but also because they originate in tissues where localized immunodepression even offers a chance for less virulent microorganisms to prevail. An implant, as a locus *minoris resistentiae*, represents an open door that creates the condition for the appearance and the prevalence of new emerging opportunistic species [23].

Biofilm

In MD-associated infections, after the initial adhesin-mediated biomaterial colonization, *S. aureus* and *S. epidermidis* produce biofilm. In biofilm, bacteria encase themselves in an extracellular material ('slime'), which embeds them together and attaches them firmly to the implant surface. The self-produced matrix consists of polysaccharides, proteins and extracellular DNA. In initial studies with coagulase-negative staphylococci the 'slime' production was considered as a virulence factor, especially in *S. epidermidis* [46,47]. Then, a high percentage of *S. aureus* strains was also found able to produce the slimy extracellular material, provided that a carbohydrate source was added in the growth medium [48–50].

Biofilm formation proceeds as a four-step process:

- Initial attachment of bacterial cells;
- Accumulation in multiple cell layers;
- Biofilm maturation;
- Detachment of cells from the biofilm into a planktonic state to initiate a new cycle of biofilm formation elsewhere [51].

The initial interactions are nonspecific and mainly hydrophobic in nature [52]. The initial attachment is mediated by specific proteins, the autolysins, first described by Heilmann *et al.* [53]. In *S. epidermidis* the autolysin/adhesin is AtlE, a 148 kDa protein [53]. In *S. aureus* the autolysin/adhesin is AtlA, a 137 kDa protein, highly homologous to AtlE [54]. Initial attachment to indwelling MDs is also mediated by MSCRAMMs, which interact with host ECM proteins filming the biomaterial surfaces [31,32].

In the second step, the intercellular adhesion is due to the production of the PIA, which is composed of linear β -1,6-linked glucosaminylglycan, whose synthesis is mediated by the intercellular adhesion (*icaADBC*) locus. This locus, initially discovered and investigated in *S. epidermidis* infections [55,56] and for a long time considered a virulence determinant, was then found to be present also in *S. aureus* [57]. A remarkable prevalence of *ica* locus in a collection of clinical isolates of *S. aureus* was documented by Arciola *et al.* [58]. Moreover the *ica* locus has been found in several other *Staphylococcus* species and DNA-sequences homologous to the *ica* locus have been detected in many coagulase-negative staphylococci [59].

Both in *S. aureus* and in *S. epidermidis* the expression of the *ica* operon and the consequent production of biofilm have been shown to depend on several environmental conditions, such as

culture media composition and supplementation [60], ethanol, salt stress [61] and iron limitation [62]. Also anaerobiosis strongly increased *in vitro* biofilm expression [63]. Not only environmental stress conditions but also bacterial density regulates biofilm production by means of signal molecules, termed autoinducers, expressed by the quorum sensing system [64]. In staphylococci the regulating system is the *agr* locus, which is activated during the transition from the exponential growth phase to the stationary growth phase by an autoregulatory mechanism involving a modified pheromone peptide that signals the state of cell density [65]. In *S. aureus* the quorum sensing system *agr* controls many virulence factors, such as the staphylococcal toxins, the adhesins to collagen, fibronectin and fibrinogen, as well as the synthesis of surface antiphagocytic capsular polysaccharide [66]. However, the role of the *agr* system on biofilm production is controversial. Basically *agr* should reduce biofilm production, but according to O'Gara the influence of *agr* in biofilm formation can vary from positive to neutral to negative, perhaps reflecting the responsiveness of the quorum sensing system to external environmental influences [67].

The production of biofilm is complex and has been shown to depend on other regulating systems, such as the gene *sar* that codes for SarA, a staphylococcal protein, which upregulates the production of biofilm by influencing the *ica* transcription and the production of poly-*N*-acetylglucosamine [68]. Another regulatory system, interconnected with the *agr* system and implicated in biofilm formation, is constituted by the target of RNAIII-activating peptide, which secretes the peptide RNAIII-activating peptide, whose increase induces biofilm growth [69].

Biofilm production can be alternatively produced via an *ica*-independent pathway, based on adhesive proteins, such as the biofilm-associated protein, discovered by the research groups of Penadés and Lasa [70,71].

Attention is now pointed to another biofilm matrix component, the eDNA [72,73], which contributes to initial attachment of *S. epidermidis* and also has a role in the accumulative phase of *S. epidermidis* biofilm formation. In *S. epidermidis*,

eDNA present in biofilm is similar to genomic DNA and has been shown to originate from lysis of a small subpopulation of bacteria, mediated by AtlE [74]. In *S. aureus* cell lysis is mediated by murein hydrolases, controlled by *cidA* and *lrgA* operons [75], which encode proteins similar to holins and antiholins, respectively, and regulate cell lysis in a manner analogous to that observed during bacteriophage-mediated cell lysis [76].

Released eDNA, as well as participating in biofilm development and stabilization, can take an important part in gene transfer mechanisms. This genetic transfer creates an opportunity for the rapid spreading of virulence as well as antibiotic-resistance genes in circulating strains exposed to the selective pressure of medical treatments [77,78].

Bacteria embedded in biofilms are resistant to antibiotics and disinfectants, to phagocytosis and other armory of the innate and adaptive inflammatory defense systems of the host [79,80].

The escape of bacteria from immune surveillance or antibiotic treatment [81,82] hampers medical treatment and requires implant removal, extensive bone debridement, excision of the infected bone and soft tissues and subsequent prolonged antimicrobial treatment [83].

Future perspective: innovative strategies to combat implant infections

Infection-resistant materials

With the aim to prevent implant-related infections, much research has been performed on the development of anti-infective and infection-resistant biomaterials, such as materials doped with effective antimicrobial drugs or resurfaced to repel bacterial adhesion and new products are continually being proposed for clinical use (Box 2). However, the concentration of bactericidal agents loaded into these biomaterials often raises the possibility of local or systemic toxicity, and the indiscriminate use of prolonged release of antibiotics leads to the potential risk of aiding the appearance of new resistant strains [84–88].

Coating the material surface with a substance able to reduce bacterial adhesion was one of the first attempts to reduce the risk of infection [89,90]. Bacterial adhesion on biomaterials may

Box 2. Innovative strategies to combat staphylococcal implant infections.

- New anti-infective, infection-resistant biomaterials and antibiotic-loaded biomaterials are being developed.
- Biofilm-dispersing enzymes: deoxyribonuclease I and dispersin B that are able to attack biofilm components appear rational and promising.
- Currently there are no commercially available anti-*S. aureus* vaccines for human infection. Microbial surface components recognizing adhesive matrix molecules may represent an ideal target for an antistaphylococcal vaccine.
- Antisense or 'antigene' molecules in the form of peptide nucleic acids able to silence or knock-out bacterial genes responsible for virulence is a possible innovative approach. However, so far peptide nucleic acids offer greater opportunities as biotechnological tools for bacterial detection and gene identification than as therapeutic agents.

be limited by modulating their hydrophilic properties with variations of polymerization processes or by modifying surfaces with surfactants [91], or by substances or drugs able to hamper the adsorption of proteins onto the biomaterial surfaces [92]. Hydroxyapatite-coated stainless steel orthopedic screws proved as an infection-resistant material [93]. Also chitosan exhibits anti-infective and, at the same time, osteointegration properties [94].

We have in the past indicated that heparin is effective in reducing staphylococcal adhesion [29,30], and we have clarified that the effect of heparin depends on specific inhibition of the binding between fibronectin, the major matrix protein, and a specific *S. aureus* adhesin [95,96].

A self-sterilizing and self-cleaning titanium dioxide (TiO₂) coating on different device surfaces has been proposed some time ago for its photocatalytic bactericidal mechanism [97] and now the antibacterial applications of TiO₂ coatings have again been investigated [98]. The interesting features of TiO₂ become even more promising when the length scale of this material is reduced to the nanometer range [99].

Among metals with antimicrobial activity, silver has raised the interest of many investigators because of its good antimicrobial action and low toxicity. Silver-based polymers have been shown to protect the inner and outer surfaces of devices against the attachment of microorganisms [100].

Aiming at using silver together with copper for their antimicrobial effect, avoiding toxic effects, nanometric thin silver and copper films covering less expensive materials has been proposed for their antimicrobial activity [101].

Nanotechnology is now offering innovative approaches: TiO₂ nanotubes incorporating silver nanoparticles seem to display long-term antibacterial ability. The TiO₂ nanotubes loaded with Ag nanoparticles were shown to kill all planktonic bacteria during the first days, and the ability to prevent bacterial adhesion is maintained steady for 30 days, a period long enough to prevent postoperation early infection and perhaps even late infection around the implant [102].

Nitric oxide is earning growing interest for its antibacterial effects: sol-gel coatings capable of nitric oxide release have recently been shown to decrease bacterial adhesion and an ingenious application is a sol-gel releasing nitric oxide coating for orthopedic implants [103].

A recent approach aimed at the treatment of mature established biofilms is photodynamic treatment, a process in which microorganisms are treated with a photosensitizer drug and then

with low intensity visible light of the appropriate wavelength. The resulting photochemical reactions generate cytotoxic reactive oxygen species, such as single oxygen and free radicals, which are able to exert a bactericidal effect [104–108].

The development of antiadhesive and anti-infective coatings on biomaterials may be expected to have a critical impact in terms of reduced risk of infection, patient morbidity, mortality and health costs.

Antibiotic-loaded biomaterials

One of the most practiced strategies to prevent and treat the development of implant-related infections is the use of antibiotic drugs. Antibiotics can be used either systemically, for prophylaxis during the perioperative phase, or locally, delivering them directly at the site of implantation. Local administration of antibiotics has been reported to offer a series of advantages, among them the avoidance of systemic toxicity and the possibility to reach active concentrations where most needed, such as at the interface between implant and tissues. Local delivery can be achieved using biomaterials preloaded in bulk with appropriate antibiotic substances, applying superficial bioactive coatings on prosthetic implant surfaces or using setting cements, gels and other highly viscous vehicle media. Current strategies of achieving convenient kinetics of antibiotic release have been further broadened by the application of nanotechnologies.

The concept of bioactive materials exhibiting antibacterial properties from the combination of biomaterials and antibiotic substances was developed in the early 1950s [109] and rapidly entered clinical use. In surgical fields presenting high risk of infections, surgeons started to treat patients with antibiotic-laden cements or antibiotic-soaked collagen sponges, even when the appearance of patented products on the market was still to come. Therefore, initial use was primarily guided by the good sense of the surgeon. However, in recent years emphasis has been given to the need for evidence-based medicine and the use of antibiotic-loaded biomaterials has been subjected to serious revisions. In a recent article we reviewed the state of the art antibiotic-loaded biomaterials, their great potential, the clinical evidence in favor of their use, and the controversies concerning their possible role in the spread of antibiotic resistance [110]. In orthopedics, the use of antibiotic-laden biomaterials for infection prophylaxis is strongly influenced by the practice of the surgeons, varying broadly in different countries, to use cemented

or uncemented prostheses. In countries in northern Europe, such as Sweden and Finland where the use of cement for implant fixation is the standard procedure, local delivery of prophylactic antibiotics is regularly practiced.

Ideal antibiotic local delivery systems for prophylaxis of implant infections should use broad-spectrum drugs, release local concentrations well above MICs, but below toxic levels, minimize the period of elution of subinhibitory concentrations, not alter the biocompatibility or interfere with tissue integration, and not evoke allergic responses. A critical prerequisite is the compatibility of the delivered substance with the biomaterial used as a vehicle. For instance, thermal stability represents a limitation for the incorporation of certain antibiotics in polymethylmethacrylate cements that are largely used in orthopedics for the fixation of prosthetic implants. Moreover, the incorporation of antibiotics can alter the physicochemical structure, finally determining a reduction in the mechanical performance of the implant [111]. However, even the complex interactions taking place at the biomaterial–tissue interface have to be taken into consideration. Thus far, most of the available delivery systems do not meet desirable release kinetics.

Virtually every known antibiotic has been explored for local delivery, from β -lactams, whose weakness is represented by low thermal stability and limited efficacy for the spread microbial resistance, to glycopeptides such as vancomycin, whose prophylactic use is regarded as highly controversial among epidemiologists. In fact, the use of glycopeptides is justifiable for only selective treatment of infections by multi-resistant microorganisms. Aminoglycosides, and in particular gentamicin, are the antibiotics that have been more broadly used for local delivery in orthopedics. However, the spread of antibiotic resistance, especially among staphylococci has significantly affected their efficacious use for prophylaxis and treatment [4,110]. Antibiotic-loaded biomaterials undoubtedly possess a high potential in medicine in the prevention and treatment of infections associated to implants, but nevertheless the growing spread of antibiotic resistances among common opportunistic pathogens represents a severe limitation. Moreover, the frequently observed long-lasting release of subinhibitory amounts of antibiotics from antibiotic-laden implants is thought to increment even further the prevalence of antibiotic-resistant strains. In light of this, future strategies should be oriented for developing anti-infective biomaterials,

exploiting bactericidal molecules alternative to known antibiotics.

Vaccines & DNA vaccines against adhesins to prevent the first attack of *S. aureus*

An ideal vaccine should induce antibodies to prevent bacterial colonization in the early stages of infection and promote opsonophagocytic killing by leukocytes. The combination of these effects should control bacterial proliferation and increase the successful attack of bacteria by the immune system.

In the search of the appropriate antigens, a variety of surface/secreted bacterial molecules that are expressed early in infection and respond to the host immune system should be isolated and analyzed. Among the antigens that have a critical role in *S. aureus* pathogenesis (early adherence to host tissues, role in bacterial growth and effects that neutralize the defense mechanisms of the host) the most promising appear to be the previously mentioned MSCRAMMs, since these adhesins are responsible for the first colonization of bacteria on the surface of implanted MDs, to which biofilm formation follows. Currently there is no efficacious anti-*S. aureus* vaccine for human infection. The complexity of the organism and the diversity of strains that cause human infection have made the development of a vaccine difficult, if not impossible. Attempts at defining a ‘core antigen’ for *S. aureus* has not yet yielded a commercially viable target.

Numerous studies have been conducted to assess whether vaccination with MSCRAMM proteins or protein fragments are protective against staphylococcal infection. In an initial study, recombinant fragments of CNA were found to be protective immunogens in a murine model of sepsis [112]. Mice vaccinated with a recombinant form of the A-region of clumping factor A exhibited a reduced arthritic response and a decrease in synovitis and joint destruction in a murine model of *S. aureus*-induced arthritis [113].

Shkreta *et al.* studied the protective measures of both clumping factor A and FnBP as a DNA-based vaccination strategy in a bovine mastitis model, obtaining a cellular and humoral immune response with a partial protection from staphylococcal mastitis and a significant reduction of inflammation and animal distress [114].

Despite the rationale that a vaccine should reach the goal to block the first adherence of staphylococci, PIA has been considered as well. Immunization with poorly acetylated PIA

conjugated to diphtheria toxin elicited opsonic killing activity against both *S. epidermidis* and *S. aureus* strains and led to a robust and protective response [115], suggesting the potential of this antigen as a broadly effective vaccine for many clinical strains of staphylococci.

The state-of-the-art approach to vaccine prophylaxis against bacterial infection is the 'DNA-vaccine'

This approach originated from 'failed' gene therapy experiments, when mice inoculated with a plasmid expressing human growth hormone elicited antibodies instead of altering growth. Tang *et al.* reported that an immune response could be elicited by introducing the gene encoding a protein directly into the skin of mice, instead of the antigenic protein itself. They concluded that genetic immunization may be time and labor saving in producing antibodies and may offer a unique method for vaccination [116].

The goal in improving immunogenic performances of DNA vaccines requires precise knowledge of cellular and molecular mechanisms by which DNA vaccines express their immunogenic potential in the human body and requires the choice of appropriate adjuvant elements as crucial molecules for strengthening DNA vaccines [117].

In implant infections caused by *S. aureus*, MSCRAMMs may represent an ideal immunogen for an anti-*Staphylococcus* vaccine. In fact, they are surface-located, are expressed at a high level both *in vitro* and *in vivo*, and play a crucial role as virulence factors. In prosthesis infections, they determine the first phase of anchorage onto biomaterials, which subsequent pathogenetic phases are dependent upon.

A survey of many studies in which staphylococcal MSCRAMMs are utilized in the design of subunit vaccines and in the development of innovative therapeutic strategies that could be implemented following the onset of infection is presented in a recent review by Arciola *et al.* From their own experience and from a critical review of the literature, the authors present a "decatalogue" for a reasoned selection of target adhesins in designing DNA vaccines against implant infections [118].

Silencing of virulence genes by peptide-nucleic-acid

The research for novel therapeutic strategies is incited by the emergence of antibiotic-resistant bacteria and the slow progresses in identifying

new classes of antimicrobial agents. The adoption of antisense or 'antigene' molecules able to silence or knock-out bacterial genes responsible for their virulence is one possible innovative approach [119,120]. The conventional approach based on antisense DNA or RNA oligonucleotides encounters several limitations both in passing through the external bacterial wall and in the rapid degradation by bacterial nucleases.

Antisense molecules that overcome these limitations are peptide nucleic acids (PNAs), in that DNA analogs in which the nucleobases are attached via methylene carbonyl linkages to a peptide chain built up by repeated *N*-(2-aminoethyl) glycine monomers instead of the sugar-phosphate backbone [121,122].

Conditions for the use of PNAs as a therapeutic drugs in silencing virulence genes are an effective delivery of this new substance into bacterial cell, and the identification and choice of an appropriate gene target.

A major problem in the utilization of PNAs in bacteria is their inefficient entry into the targeted pathogen due to the outer membrane barrier. A solution to this problem has come from the knowledge of the mechanism of entry bacterial wall by human defensins or other cationic peptides, such as those expressed by neutrophils, which are able to penetrate and disrupt bacterial membranes.

Good *et al.* suggested that attachment of a leader cationic peptide to the PNA molecule favors bacterial entry and proposed the amino acid sequence KFFKFFKFFK, attached as a leader peptide to a short PNA (10–12 bases) [121]. This expedient facilitated entry into *E. coli*, creating a potent antimicrobial effect [123,124].

The reason for the efficacy of this leader peptide and for its usefulness in bacterial entry is its amphipathic structure: the positively charged residues (lysine) preferentially interact with the anionic phospholipids present in the bacterial external membrane (eukaryotic cell membranes are instead characterized by a high content of dipolar phospholipids) and the nonpolar amino acid side chains (phenylalanine) favor attachment to the bacterial external lipid layer.

With regard of the choice of target gene, inhibition of gene expression and of bacterial growth have been achieved in *S. aureus* by targeting the essential genes *fmbB* (involved in cell wall biosynthesis), *hmrB* (high methicillin resistance gene), *gyrA* (involved in DNA replication) and *acpP* (involved in fatty acid biosynthesis) with PNAs [125,126].

The aforementioned examples indicate that PNAs are efficacious agents for inhibiting bacterial growth and for interfering with experimental infections, but silencing or knocking-out bacterial genes by antisense PNAs; however innovative and fascinating, has not yet provided the 'magic bullet' in combating bacteria responsible for implant infections. Although PNAs have been shown to interfere with the expression of basic genes for bacterial growth and with the expression of antibiotic resistance genes, they, so far, offer greater opportunities as biotechnological tools for bacterial detection and gene identification than as therapeutic agents.

Conclusion

Projections based on the actual trend indicate that the number of arthroplasties is destined to increase in the next decades and implant infections remain the major and often irreducible complication in the clinical use of biomaterials, especially in orthopedics, demanding new therapeutic and preventive strategies. The study and comprehension of virulence factors, their relative weight and their regulatory mechanisms can support new strategies, also at the molecular level, as gene therapies aimed at silencing or knocking out genes responsible for the bacterial implant colonization (adhesins, biofilm production and antibiotic resistance).

Executive summary

Virulence factors & pathogenesis of implant infections

- Implant-related orthopedic infections are typically caused by opportunistic pathogens taking advantage of the presence of foreign bodies that condition the level of the immune defense of host tissues in the immediate proximity.
- The most common bacteria isolated from infected joints are staphylococci. *Staphylococcus aureus* is the main opportunist pathogen implicated in implant-related infections in most surgical fields, the orthopedic one among them.
- The infecting organisms are either introduced during implantation of a prosthesis or derived from a temporary bacteremia.
- Staphylococci adhere to biomaterials and grow to form a biofilm. The biofilm offers enhanced protection against natural host defenses and antibiotic therapies.

Role of adhesins in the first step of infection

- Adhesins represent an important group of virulence factors mediating surface adhesive interactions between microbial cells and between these and host cells and the extracellular matrix. They play a primary role, particularly in the early phases of infection pathogenesis of tissue tropism, surface adhesion and colonization.
- The adhesins group includes the cell wall-anchored microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) as well as the more recently described secreted expanded repertoire adhesive molecules (SERAMs). MSCRAMMs, in this differing from SERAMs, are receptorial proteins anchored to the bacterial cell wall through a typical cell wall sorting signal Leu-Pro-X-Thr-Gly (LPXTG) motif.
- The most epidemic and successful clone of *S. aureus* responsible for biomaterial-associated orthopedic infections is endowed with both bone sialoprotein-binding protein and collagen-binding protein, two adhesins whose respective ligands represent by far the main bone extracellular matrix protein components.

Biofilm

- In medical device-associated infections, after adhesin-mediated initial colonization onto biomaterial, *S. aureus* and *Staphylococcus epidermidis* make a use of another virulence factor: the biofilm.
- The major biofilm component is the polysaccharide intercellular adhesin, composed of linear β -1,6-linked glucosaminylglycan, whose synthesis is mediated by the intercellular adhesion (*icaADBC*) locus.
- Besides polysaccharide intercellular adhesin, biofilm contains proteins and extracellular DNA.

Innovative strategies to combat Staphylococcal implant infections

- With the aim to prevent implant-related infections, anti-infective and infection-resistant biomaterials are being developed. New products are continually being proposed for clinical use, among which are antibiotic-loaded biomaterials.
- Among many agents taken into consideration for staphylococcal biofilm disruption, enzymes able to attack biofilm components appear rational and promising. Two well-studied biofilm-dispersing enzymes are deoxyribonuclease I and dispersin B.
- Currently there are no commercially available anti-*S. aureus* vaccines for human infection. In implants, infections caused by *S. aureus* MSCRAMMs may represent an ideal target for an antistaphylococcal vaccine. In fact, they are surface-located, are expressed at a high level both *in vitro* and *in vivo* and play a crucial role as virulence factors. In prosthesis infections, they determine the first phase of anchorage onto biomaterials, which all the other pathogenetic phases are dependent upon.
- The research for novel therapeutic strategies is incited by the emergence of antibiotic-resistant bacteria and the slow progress in identifying new classes of antimicrobial agents. The adoption of antisense or 'antigene' molecules able to silence or knock-out bacterial genes responsible for virulence is a possible innovative approach. Antisense molecules in the form of peptide nucleic acids have been shown to interfere with the expression of basic genes for bacterial growth and with the expression of antibiotic resistance genes, but so far, peptide nucleic acids offer greater opportunities as biotechnological tools for bacterial detection and gene identification than as therapeutic agents.

Development of immunological approaches (blocking early bacterial adhesion) or the use of enzymes that interfere with the biosynthesis or the activity of specific biofilm components or able to disrupt formed biofilms may prove to be useful in preventing or treating infections compromising the success of MDs [127]. However, because staphylococcal infections involve multiple virulence determinants, it is unlikely that a single strategy against a specific target will protect the host from the infection. The synergistic use of immunotherapy or drugs in combination with traditional or new antibiotics

could broaden the clinical options and improve the effectiveness of antimicrobial therapy.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Website

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www.njrcentre.org.uk