DRUG DISCOVERY TODAY TARGETS



# *Staphylococcus aureus*: the search for novel targets

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**In the UK, 20,000 cases of *Staphylococcus aureus* bacteraemia are reported each year, half of which are antibiotic resistant and ~4% are fatal, exemplifying a worldwide phenomenon of tremendous economic and human impact. Novel treatments and prophylaxis are urgently required to combat such a serious threat. A common goal in the postgenomic era is to identify new targets for drug intervention (using small molecules) and immunologicals. Several promising cellular targets are now being developed in the quest to control such a life-threatening pathogen.**

*Staphylococcus aureus* is the pathogen most frequently isolated worldwide from community- and hospital-acquired infections of the bloodstream, lower respiratory tract, skin and soft tissue [1]. It is the causative agent of a variety of diseases in animals and humans ranging from sub-acute superficial skin lesions to life-threatening septicaemia [2,3]. Infection is often associated with wounds or surgical procedures resulting in a localized abscess from which systemic disease can spread. *S. aureus* has also been notoriously successful at developing antibiotic resistance. The number of methicillin-resistant *S. aureus* (MRSA) in hospital-acquired infections has increased in recent years by 10–15% in countries such as Germany, the UK and the USA [4,5]. Moreover, the trend observed in hospitals might be extending to the community [6]. The problems with *S. aureus* are exacerbated because it is almost ubiquitous in the human environment. *S. aureus* is carried by ~30% of healthy humans, most commonly in the anterior nares, which provides a ready reservoir for infection [7]. Recently, the spectre of untreatable infections has arisen as resistance even to the 'antibiotic of last resort' (vancomycin) has appeared in the clinic [8]. Over the years, many new drugs have been developed and are continuing to be launched on the market against existing, well-characterized targets. Examples of such drugs include dalbavancin, a glycopeptide antibiotic that interferes with peptidoglycan (PGN) biosynthesis via a similar mode of action to vancomycin (www.vicuron.com/products/proprietary/dalbavancin.html), and gemifloxacin, a quinolone affecting DNA replication (www.factive.com). However, it is probable that the characterization of novel targets will lead to the discovery of new chemical classes of inhibitors, against which resistance will take longer to appear in the clinical setting. Thus, to meet the growing challenge of *S. aureus*, several approaches are being undertaken to develop new prophylaxis and therapy. These include the identification of novel targets for small molecule inhibitors, enzymes and immunologicals. Here, representative current developments for the control of this important human pathogen are highlighted.

## Good drug targets: essential gene products and how to find them

Gene products that are essential for cell growth under all conditions are primary targets for therapy because their inhibition would be fatal for the cell. For example, the inhibition of DNA gyrase by ciprofloxacin prevents DNA replication. Thus, classic and novel techniques to study essential cellular components have potentiated the road to drug discovery. The

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**GLOSSARY**

**Comparative genomics**: Prediction of genome structure and function based on comparison with other genomes.
**Conditional lethal mutation**: Mutation accomplished by exogenously controlled gene expression.
**Immunologicals**: Antibody-based therapeutics.
**Opsonophagocytosis**: Phagocytosis induced by opsonins (i.e. antibodies and particular complement fragments) that bind antigens on bacterial surfaces and receptors on neutrophils and macrophages.
**Pathogenicity islands**: Sections of a bacterial pathogen genome that contain clusters of genes required for virulence.
**Quorum sensing**: Cell-density-dependent signalling process.
**Sensor–regulator**: Conserved bacterial environmental sensing and signal transduction mechanism.
**Serotype**: Bacterial strain classified by a specific host antibody response.
**Superantigen (SAgs):** Staphylococcal proteins responsible for overstimulation of the immune system during infection.
**Virulence factor**: Bacterial component required for disease.

now available multiple genome sequences of *S. aureus* have revealed the entire genetic code, including essential genes [3,9,10] (www.sanger.ac.uk/Projects/S_aureus; www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gsa). The problem that must be tackled now is distinguishing essential genes from the rest. Comparative genomics, using data compiled from other organisms, has proved useful in the initial designation of putative gene identity, including essential gene components [3,9,10].

Genes within a cell that are refractory to deletion or insertional inactivation are likely to encode essential functions. This was the basis of a comprehensive study undertaken in *Bacillus subtilis* to define the role of all the genes in the chromosome by targeted mutagenesis. This systematic 'gene-by-gene' approach identified essential gene candidates [11]. These were further analysed through the generation of conditional lethal mutations based on the controlled expression of the chosen genes by a regulatable promoter (engineered to replace the wild-type promoter). This study revealed 271 genes (~7% of the chromosome) crucial for growth of *B. subtilis*, most of which have homologues in *S. aureus*. Indeed, 52% of all the predicted proteins in *S. aureus* are conserved in *B. subtilis* [9].

In *S. aureus*, similar conditional lethal constructs have been used to verify the essentiality of several components, including polypeptide deformylase (Pdf1) [12], the cell wall acetylmuramyl tripeptide synthetase (MurE) [13,14] and the PGN pentaglycine interpeptide synthetase (FmhB) [15], all of which have been considered attractive targets for future antimicrobials (Figure 1). Alternative technologies for the identification of essential genes have also been developed for *S. aureus*. The *murB* gene (the product of which is involved in PGN biosynthesis) has been isolated as a temperature-sensitive mutation (where growth only occurs at the permissive temperature) [16]. Inhibitors of MurB activity have already been developed (Table 1) [17–20].

Another technology to identify essential gene products is based on the regulatable expression of antisense RNA molecules, which interfere with the translation of the corresponding cognate genes [RNA interference (RNAi)]. This technology has been applied to *S. aureus* as a shotgun approach to analyse the whole genome comprehensively, identifying up to 658 genes, many of which are conserved and are essential in other bacteria [21,22]. It is important to bear in mind that this technology can

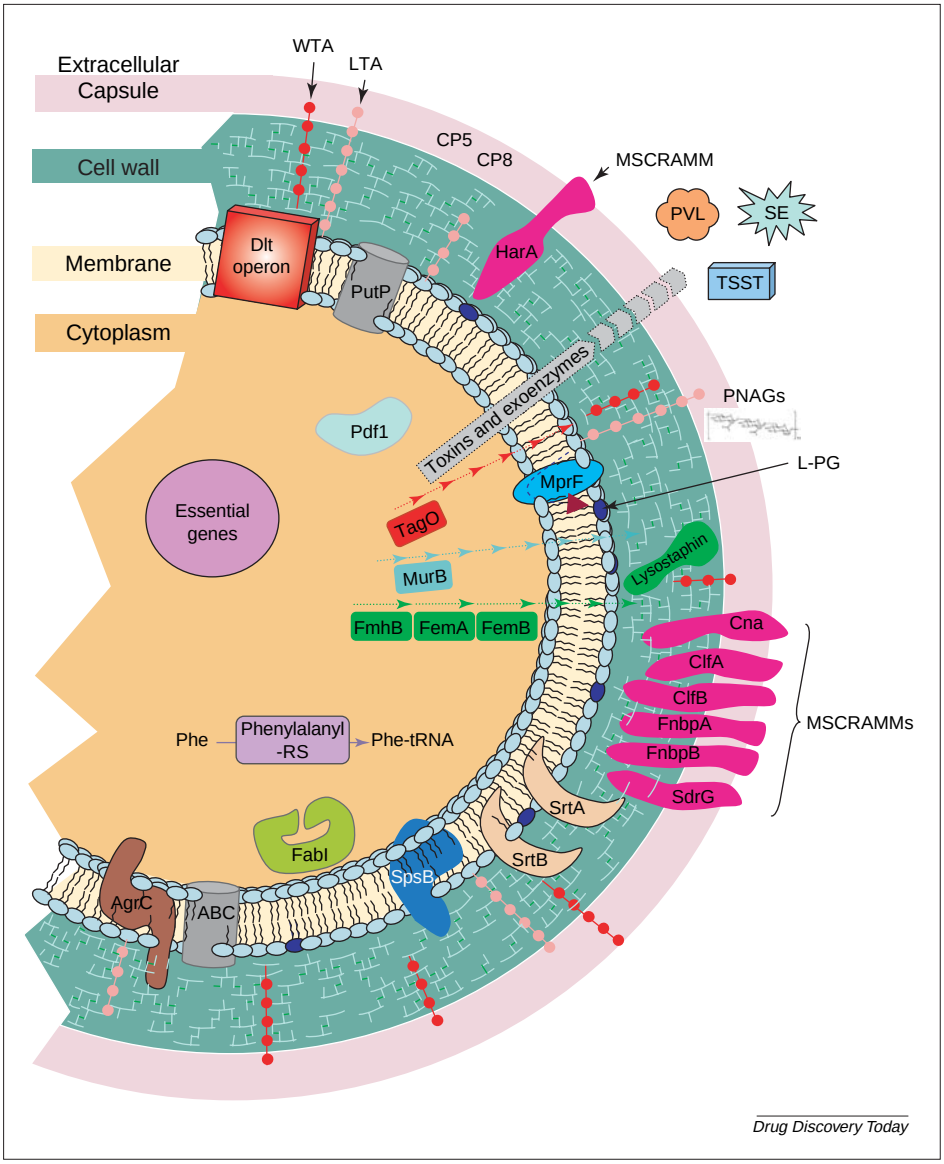


**FIGURE 1**

**Novel antimicrobial targets in *Staphylococcus aureus*.** The components are distributed according to their corresponding sub-cellular localization.

**TABLE 1**

**Inhibitors of novel *Staphylococcus aureus* targets**

| Cellular compartment | Target | Identity | Role | Mode of targeting | | Refs |
|---|---|---|---|---|---|---|
| | | | | Inhibitor or enzyme | Immunological | |
| Extracellular | SE | Enterotoxin Superantigen | Food poisoning Shock | DRα1-linker–TcRVβ chimera | | [33] |
| | TSST | TSST Superantigen | TSST | DRα1-linker–TcRVβ chimera | | [33] |
| | PVL | PVL pore-forming toxin | Skin and lung infection | | Anti-PVL antibody | [36] |
| Capsule | CP5 CP8 | CP | Attachment to surfaces and immune evasion | | StaphVax vaccine AltaStaph (passive immunization) | [38,39] |
| | PNAGs | Surface polysaccharide | Attachment to surfaces | | Active and passive immunization | [44] |
| Cell wall | Pentaglycine cross bridge | PGN structure | Maintenance of cellular integrity | Lysostaphin (endopeptidase) | | [53–56] |
| | Cna, ClfA and FnbpB fragments | Ligand binding | Adhesins<br>Host–pathogen interaction | | Active and passive immunization | [57,60,61] |
| | ClfA, SdrG and other MSCRAMMs | Ligand binding | Adhesins<br>Host–pathogen interaction | | Veronate® (anti-ClfA, anti-SdrG and others; passive immunization)<br>Aurexis® (humanized anti-ClfA; passive immunization) | Inhibitex; [60] |
| Membrane | PutP | High-affinity proline permease | Amino acid transport | Proline analogues – L-azetidine-carboxylic acid and 3,4-dehydro-D,L-proline | | [28] |
| | SrtA | Cysteine-protease transpeptidase | Linking of MSCRAMMs to cell wall PGN | Methanethiosulfonates and *p*-hydroxymercuribenzoic acid<br>Diazomethane<br>Chloromethane<br>Benzyloxycarbonyl substrate-derivatives<br>Phosphinic peptidomimetics | | [68]<br>[70]<br>[69] |
| | SpsB | Signal peptidase | Protein secretion | Lipopeptide<br>α-Ketoamides | | [71,74] |
| | ABC transporter (SA1224 in *S. aureus* N315) | Solute transporter | Unknown | | Aurograb® (anti-ABC epitopes; passive immunization) | Neutec Pharma; [75] |
| | AgrC | Receptor histidine kinase | Regulation of virulence genes (two-component quorum-sensing system) | Truncated autoinducer<br>Peromonycins<br>(AIP-fused colicin) | | [80]<br>[81] |
| Cytoplasm | Pdf1 | Polypeptide deformylase | N-terminal formylation of ribosome-synthesized polypeptides | Hydroxamic acid derivatives | | [12] |
| | MurB | UDP-*N*-acetylmuramate dehydrogenase | PGN biosynthesis | 4-Thiazolidinones<br>Imidazolinone analogues<br>Pyrazol-3-ol derivatives<br>Thiazolylurea derivatives<br>Carbamate derivatives | | [17]<br>[20]<br>[19]<br>[18] |
| | FabI | Enoyl-ACP reductase | Lipid biosynthesis | Naphthyridin acrylamide derivatives | | [86] |
| | Phenylalanyl-RS | Phenylalanyl-tRNA synthetase | Protein biosynthesis | Phenyl-thiazolylurea-sulfonamides | | [84] |

provide false negatives if the controlled expression of the antisense RNA molecule is not enough to counteract the amount of mRNA corresponding to the cognate essential gene. False positives can also arise as a result of non-specific RNA interaction, which could explain the high number of putative essential genes identified with this technology. Antisense RNA technology as a means for therapy, which has been explored extensively in molecular medicine, has been recently adapted for the inhibition of *S. aureus* protein expression, thereby validating its application as a suitable anti-staphylococcal treatment [23]. In this case, the gene knockdown was enabled by exogenous administration of antisense peptide nucleic acids (PNAs), which are DNA mimics with a pseudopeptide backbone that are able to form stable duplex structures with cDNA or RNA. Thus, a combination of multiple approaches is proving successful in defining the exact complement of essential gene products in *S. aureus* that could potentially be novel drug targets.

## Pathogenicity-associated components: *in vivo* technology

The targeting of bacterial components expressed during, and important for, infections (pathogenicity-associated components) is a potential strategy to combat staphylococcal disease. Although this strategy might not kill the pathogen directly, it could debilitate it such that the immune system will give effective clearance, which has the potential advantages of not affecting the resident microflora and not readily giving rise to resistant strains. However, drugs are likely to be narrow spectrum and drug development will be difficult because it would be problematic to assess inhibition *in vitro*.

The *S. aureus* genome sequences have revealed many putative virulence determinants, including several clustered on 'pathogenicity islands' [3,9]. Several methods have been applied for the identification of pathogenicity-associated components, for example, *in vivo* expression technology (IVET), which relies on a promoter trap system [24]. In effect, IVET is able to identify those bacterial genes that are specifically expressed only during infection. This technology has resulted in the identification of several genes, including the accessory gene regulator (*agrA*), which is responsible for the regulation of more than 20 virulence factors in *S. aureus* [25], therefore validating the applicability of this technology in this bacterial species. AgrA controls a quorum-sensing system that could be a useful target for inhibition.

Signature-tag mutagenesis (STM) is able to identify bacterial genes required for disease. A pool of mutants (each specifically tagged) is used to infect an animal. Tagged mutant pools are compared before and after infection and those strains that are not represented in the output pool are deemed attenuated and the tagged gene important in pathogenesis. In *S. aureus*, STM has identified several virulence determinants [26,27], including *putP*, which encodes a high-affinity proline transporter. In addition, the use of proline analogues as bacteriostatic inhibitors of proline uptake rendered promising results in the control of *S. aureus* [28]. More recently, an STM refinement has identified a further pool of virulence factors that could be useful targets [29].

Pathogenicity-associated components exposed on the cell surface are potentially targets for immunological prophylaxis and therapy. Protein surface display technology has been used to identify *in vivo*-expressed vaccine candidate antigens from *S. aureus*. A total of 60 antigenic proteins were identified, the majority of which were either expressed on the surface of *S. aureus* or secreted [30], including the iron-regulated surface haptoglobin receptor A (HarA) (Figure 1) [31]. HarA binds to the human iron-carrier molecule haptoglobin (and also haptoglobin–haemoglobin complexes) with the probable function of supplying *Staphylococcus* with iron, a metal precious for life and difficult to access in living systems. The inhibition of bacterial iron acquisition has long been researched for novel therapeutic options. Overall, *in vivo* protein surface display is limited, for example, to the expression of non-toxic peptides. Another technology, ribosome display, overcomes that limitation and its use has resulted in the identification of 14 putative novel short open-reading frames within the *S. aureus* genome that encode antigenic polypeptides [32]. However, this technique can also overestimate the number of surface components, because some of the gene products identified corresponded to those previously annotated as cytoplasmic. Finally, a potential problem with the analysis of *in vivo*-expressed components as antigens is that levels of achievable antibody response might not be protective.

## From drug target to control

There is a plethora of potential targets in all cellular compartments, ranging from essential cytoplasmic enzymes as drug targets to surface proteins for immunotherapy. However, the translation from potential to reality is acutely difficult.

### *Toxins and exoenzymes*

More than half the known virulence determinants in *S. aureus* correspond to extracellular enzymes and toxins [9] and their neutralization seems a reasonable primary objective for therapy. Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) bind to the D region α (DRα) domain of the major histocompatibility complex (MHC) class II receptor on antigen-presenting cells (APCs) and the Vβ domain of the T cell receptor (TcRVβ) (Figure 1). This binding acts as a powerful mitogenic signal to these cells and induces a substantial release of cytokines, such as interferon (IFN)-γ, which can result in shock and death [23]. DRα1-linker–TcRVβ chimeras targeting SE-B, SE-C3 and TSST-1 exhibit superantigen-specific inhibition (Table 1) [33]. These chimeras represent a promising therapeutic tool, which needs to be improved by increasing

the binding affinities and removal of the cross-reactivity with the various TcRVβ isoforms. An alternative approach is vaccination with mutated non-toxic TSST-1, which improves survival of immunized mice. This effect appears to be mediated by neutralizing antibodies to TSST-1 and a concomitant decrease in cytokine production *in vitro* [34]. The Panton–Valentine leukocidin (PVL) is proposed to be a primary virulence factor in pulmonary necrosis in staphylococcal pneumonia [35]. Studies have shown neutralization of PVL by anti-PVL antibodies *in vitro* [36], and thus antibodies against PVL could have therapeutic value (Table 1) [25].

### *Capsule and extracellular polysaccharides*

The outermost layer covering *S. aureus* comprises capsular polysaccharides (CPs), thus enabling resistance to opsonophagocytosis and persistence in the nares [37]. The capsule is present in the majority of *S. aureus* strains, with serotypes CP5 and CP8 being the most prevalent among human isolates (Figure 1). Expression of CP5 and CP8 is induced *in vivo* in vertebrate models of pathogenesis (as detected by STM or IVET technologies) [24,26].

CPs are poorly immunogenic and normal antibody levels to CP5 and CP8 are too low to afford protection. However, the immunogenicity is increased using CP conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) and various adjuvants (Table 1) [38,39]. However, clinical trials up to Phase III based on promising data in mice vaccinated with combined CP5- and CP8-exotoxin A conjugate variants (StaphVax) did not show significant efficacy in humans [38,39]. Currently, purified antibodies (AltaStaph) against CP5 and CP8 are in clinical trials (www.nabi.com/pipeline/pipeline.php?id=2).

The ability of *S. aureus* to adhere to inert surfaces (e.g. medical devices) and colonize living tissue, such as cartilage and heart valves, can be partially explained by the formation of intercellular aggregates and biofilms: biofilm formation also increases evasion of host defences and tolerance to antibiotics [40]. The polysaccharide intercellular adhesin (PIA) [41] and CP/adhesin (PS/A) [42] of *S. aureus* belong to the class of poly-*N*-acetyl-glucosamine (PNAG) antigens and are attractive targets for antimicrobial development (Figure 1) [43]. First attempts at PNAG immunization revealed that it was moderately protective in rabbit models of endocarditis and catheter-associated bacteraemia, as well as in a murine kidney infection model (Table 1) [44]. Passive immunization with anti-PNAG sera and monoclonal and polyclonal antibodies also offers a degree of protection (Table 1).

### *Teichoic acids: a shared feature of the cell wall and membranes*

Teichoic acids (TAs) are polyglycerol phosphate or polyribitol phosphate polymers decorated with sugar substituents or esterified with D-alanyl groups, and anchored to either the cytoplasmic membrane [lipoteichoic acids (LTAs)] or the cell wall PGN [wall teichoic acids (WTAs)] (Figure 1) [45]. The positively charged free amino group of the D-alanyl residues neutralizes the anionic properties imparted by the phosphate groups, protecting *S. aureus* from attack by cationic antimicrobial peptides (CAMPs) of the host innate immune response. The positively charged amino groups enhance biofilm formation, and are required for full virulence in a mouse model of infection [43,45–47]. TA alanylation of both LTA and WTA is undertaken by DltA, DltB, DltC and DltD (Figure 1). These have been proposed as potential drug targets by Neuhaus [45] because the deficiency of ester formation increases the sensitivity of *S. aureus* to host-generated defences and would decrease the immunostimulatory properties of LTA [45]. It was also suggested that D-alanine analogues could interfere with the activation and transfer of D-alanyl residues to prevent TA substitution. The first enzyme of the WTA assembly pathway (TagO) could also constitute a suitable target for antibiotic development because, although not essential for *S. aureus* growth, it is required for nasal colonization, which is a predisposing factor for nosocomial infections (Figure 1) [7,48].

The resilience of *Staphylococcus* to CAMPs is also conferred by one of the major membrane phospholipids, a derivative of phosphatidylglycerol modified with L-lysine – lysylphosphatidylglycerol (L-PG) (Figure 1) [49]. Because there are two free amino residues in the lysyl group, the molecule has a net positive charge, and is therefore less likely to interact with CAMPs. MprF is necessary for the synthesis of L-PG and an *mprF* mutant is virulence-attenuated in a mouse model of sepsis, confirming that CAMP resistance is most probably an important virulence factor in *S. aureus* (Figure 1) [49].

The exploitation of naturally occurring CAMPs is another avenue currently being explored for the treatment of staphylococcal infections. However, CAMPs are often salt-sensitive, have weak antimicrobial activity and are cytotoxic or cytolytic to eukaryotic cells. Efforts have been directed towards the synthetic enhancement of their potency, while reducing toxicity and improving pharmacokinetic properties. Indeed, derivatives of magainins, which are CAMPs isolated from frog skin, have already passed clinical trials [50].

### *Cell wall: peptidoglycan*

PGN is unique to eubacteria, including *S. aureus*, and inhibition of its production is generally bactericidal (Figure 1). Inhibitors of PGN biosynthesis are classically some of the most important groups of antibiotics ever developed (e.g. glycopeptides, β-lactams and phosphomycin).

MurB acts early in PGN biosynthesis and has recently been shown to be essential for the growth of *S. aureus* [16], a finding that suggests MurB could be a suitable new target for drug development (Figure 1). *S. aureus* PGN is characterized by a pentaglycine bridge, which is essential for the structural integrity of the macromolecule. Thiazolidinone

surrogates of the UDP-sugar moiety of the MurB substrate were developed based on the X-ray crystallographic data of MurB (Table 1) [17]. The formation of the pentaglycine bridge is catalysed by the FemABX peptidyl transferase system: mutants of *femAB* are barely viable and *femX* (*fmhB*) is essential for life, therefore they are attractive targets for drug development (Figure 1) [15,51]. Lysostaphin is an endopeptidase that cleaves the pentaglycine cross-bridges in the cell wall. Its native form has been successfully used for the eradication and/or prevention of *S. aureus* colonization of nares and other living tissues, in addition to inert materials, such as catheters (Table 1) [52]. Moreover, lysostaphin successfully treated endocarditis in a rabbit model of infection [53,54]. When expressed in transgenic mice, it enhanced resistance against *S. aureus* mastitis (Table 1) [55,56]. Thus, enzyme therapy could be useful in the reduction of nasal carriage, as well as the treatment of disease.

### *Cell envelope-bound proteins*

The attachment of *S. aureus* to surfaces initiating the colonization process is mediated by several cell wall-associated proteins, the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (Figure 1). The best characterized MSCRAMMs are the fibronectin-binding proteins (Fnbp) FnbpA and FnbpB, the collagen-binding protein Cna and the fibrinogen-binding proteins clumping factor (Clf) A and ClfB (Figure 1). ClfA is a virulence factor in a septic arthritis mouse model and ClfA and ClfB promote adhesion to vascular and endocardic lesions [57,58]: ClfB also mediates adherence to the nasal epithelia [59]. Fragments of Cna, ClfA and FnbpB are protective immunogens, when used as vaccines in rodent models of infection, reducing the severity of the symptoms, decreasing mortality between twofold and sixfold and/or preventing dissemination (Table 1) [57,60,61]. Passive immunization with human anti-ClfA polyclonal antibodies, rat anti-FnbpB or rat anti-Cna enhanced survival in murine models of infection (Table 1) [60,61]. Veronate® (Inhibitex), a cocktail of antibodies including polyclonal anti-ClfA and anti-SdrG (an *S. epidermidis* protein) from selected human donors, is currently undergoing clinical trials to treat staphylococcal infections in low-weight birth neonates (Table 1) [60]. Similarly, a monoclonal humanized anti-ClfA antibody (Aurexis®; Inhibitex) protects approximately half the population of assayed mice against *S. aureus* infection and is currently in Phase II clinical trials (Table 1) [60].

A DNA-based vaccine using a plasmid encoding a binding portion of ClfA increased the titre of anti-ClfA antibodies in mice, resulting in decreased fibrinogen binding and stimulation of phagocytosis. However, *S. aureus* opsonized with this serum showed only a moderately decreased invasion of mammary glands in a mouse mastitis model, and DNA-vaccinated mice were not protected when intraperitoneally injected with *S. aureus* [62]. Similarly, when used in cows, this vaccine seems to decrease the bacterial shedding in milk by less than twofold [63]. Combinations of DNA-immunization and active–passive protein-immunization could overcome the prevention of infection insufficiency derived from vaccines against single MSCRAMMs, as well as increase the *in vivo* efficacy detected thus far.

### *Membrane*

Membrane interacting antibiotics already exist, for example, the lipopeptide daptomycin, but new avenues are also being explored. The linking of proteins to the cell wall PGN, including the MSCRAMMs, is catalysed by membrane-anchored cysteine protease-transpeptidases (i.e. the sortases SrtA and SrtB) through the recognition of sorting signals within the cognate proteins (Figure 1) [64]. Given the contribution of many of the MSCRAMMs in *S. aureus* virulence, sortases are attractive targets for antimicrobial development. SrtA is required for the original establishment of infection and SrtA and SrtB for persistence in various animal models [65–67]. The crystallographic structure of both sortases is now known and could aid in the process of custom drug design [68]. *S. aureus* SrtA and SrtB have different binding pockets for their corresponding substrates. The comparison between the structures provides insight into substrate specificity that can be used to design suitable analogue inhibitors [69,70]. Already, several independent classes of sortase inhibitors have been described and are under development (Table 1).

Protein precursors destined for secretion are processed by signal peptidases (SPases), which cleave off the signal (or leader) peptide (Figure 1) [71]. A conditional lethal gene construct for type I signal peptidase (SpsB) has demonstrated its essentiality in *S. aureus* [72]. SpsB is also a strong candidate for specific inhibitor development because of its accessibility and prokaryotic exclusivity. Inhibitors targeting bacterial SPase I have been extensively researched, resulting in the identification of novel (5*S*)-penem compounds (a class of β-lactam antibiotic) and lypoglycopeptides [71,73]. Lipopeptide α-ketoamide inhibitors tailored to the *S. aureus* SpsB are currently under investigation (Table 1) [71,74].

An immunodominant antigen in MRSA infections is part of a membrane-associated ATP-binding cassette (ABC) transporter (Figure 1) [75]. In rabbits, recombinant antibodies against peptides based on the ABC subunit of the ABC transporter reduced bacterial counts in selected organs compared with non-immunized controls. This immunotherapeutic approach has been further developed as Aurograb® (Neutec Pharma), which is currently undergoing clinical trials (Table 1).

The *agr* locus is a global sensor–regulator of virulence genes in *S. aureus*, including the genes encoding the α- through δ-haemolysins and other exotoxins, cell wall-bound MSCRAMMs, including ClfA and SdrC, TA biosynthesis, and exoenzymes such as staphylokinase and lipase. AgrC and AgrA form a sensor–regulator pair that control gene expression in response to environmental stimuli and are

required for pathogenesis in several models (Figure 1) [76–79]. AgrC and AgrA respond to a quorum-sensing mechanism dependent on an autoinducer peptide (AIP), which is itself a product of another gene within the *agr* locus. In several models, inactivation of this system has been undertaken through the rational design of an inhibitor based on the truncated version of one of the naturally occurring AIPs [80]. Furthermore, colicins have been targeted to AgrC through fusions with the AIPs (pheromonycins) for their deployment in *S. aureus* (Table 1) [81]. The regulation of virulence determinant production has also been targeted in animal graft models by chimeric AIP peptides, which prevented *S. aureus* biofilm formation [82,83].

### *Cytoplasm*

Classical antibiotics against cytosolic components, such as ribosomes (e.g. aminoglycosides and tetracyclines), are well-known and have been widely used. Resistance towards them has prompted the search for new alternative targets. Phenylalanyl-tRNA synthetase (Phenylalanyl-RS) is an essential enzyme that catalyses the transfer of phenylalanine to Phe-specific tRNA (tRNAPhe), a key step in protein biosynthesis (Figure 1). HTS and chemical variation of the screening hit led to the identification of phenyl-thiazolylurea-sulfonamides as a novel class of potent inhibitors of bacterial Phenylalanyl-RS (Table 1) [84]. These compounds inhibit the Phenylalanyl-RS of *Escherichia coli, Haemophilus influenzae, S. pneumoniae* and *S. aureus*.

The enoyl-acyl carrier protein (ACP) reductase (FabI) performs the last step in the fatty acid biosynthetic pathway (Figure 1). In *S. aureus* and *E. coli*, this enzyme is the target of the inhibitors triclosan and diazaborines [85]. However, FabI is not present in *Streptococcus pneumoniae*, which has another enzyme responsible for performing the same function, FabK: FabI and FabK are found in some pathogens, for example, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Consequently, FabI represents a selective antibacterial target for those pathogens, such as *S. aureus*, wherein FabI is the sole enoyl-ACP reductase. New classes of FabI-directed antibacterials with clinical potential have been reported (Table 1) [86].

Interestingly, studies in different animal models, *in vivo* expression studies and genome-wide knockout libraries with *S. aureus* have shown that the majority of genes expressed, involved or required for pathogenesis belong to the metabolic gene class [22,26,27,87]. The identification of nutrient sources preferentially used during infection will enhance the refinement of drug design strategies [88]. However, it will be important to define nutritional requirements that are important across the range of *S. aureus* infections and not specific for a particular host niche.

## Concluding remarks

A recent article by Zalacain and co-workers [14] analysed, by regulatable allelic replacement, the essentiality of genes encoding proteins of unknown function in various genomes, including *S. aureus*, as well as compiling data from other studies [11,22]. Interestingly, six of the 22 genes identified as essential in *S. aureus* (within a total of 34 replacements) were not essential in *B. subtilis*. Indeed, only four of the 144 genes studied seemed to be consistently essential in most of the species evaluated (*S. pneumoniae*, *S. aureus*, *B. subtilis*, *H. influenzae* and *E. coli*), namely, *ydiC* and *ydiE* (putative glycoproteases), *ylqF* (putative low molecular weight GTPase) and *ykqC*. Whether these genes will be useful drug targets remains to be established. For proteins of unknown function, drug development could be hampered by a lack of biochemical assays. Ongoing structure–function studies will not only reveal the roles of these important proteins but will also facilitate the rational design of inhibitors as a first step in drug development.

Although many of the cell envelope-associated proteins appear as appealing targets, particularly for immunological approaches, because of their exposed location and virulence involvement (e.g. ClfA and FnbpB), their efficacy has yet to be proven. The history of drug development and the inevitable appearance of resistance render it crucial to develop immunological approaches as alternatives to small molecule inhibitors. These could take the form of vaccines, prophylactic or therapeutic antibodies.

The advent of full genome mutant libraries augers well for the role of all genes in pathogenesis to be determined [87]. With the rapid advances in post-genomic technologies in the near future, the entire complement of *S. aureus* genes essential *in vitro* and important *in vivo* will have been identified. How this knowledge is used in the development of new drugs or immunological approaches is difficult to predict. Several different targets are being pursued for vaccine development but they might not be of general use. Thus, it is imperative to continue a diverse portfolio of complementary approaches to prevent the spectre of untreatable infections.

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