Staphylococcal Infection, Antibiotic Resistance and Therapeutics

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1. Introduction

Staphylococcus spp. are a challenge for the modern day medicine due to the complexity of disease process and presence and expression patterns of their respective virulence factors. The members of this genus possess many known toxins, multiple immunoavoidance mechanisms and adherence factors, most of which demonstrate transient, timed, and disease-specific expression. They cause different types of infections in a host that are either planktonic, biofilm mediated or both. Sepsis and pneumonia are mainly caused by planktonic forms whereas, a whole range of diseases, namely, endophthalmitis, osteomyelitis, endocarditis, chronic skin infections, indwelling medical device infections, chronic rhino-sinusitis, and dental implantits are caused by the biofilmic form of the bacteria. Abscess can be caused by both of the forms (Harro et al., 2010). Staphylococci are human pathogen, known for their ability to become resistant to antibiotics. They have been associated, besides causing ophthalmic infections, with skin infections and sepsis. Methicillin resistant *S. aureus* (MRSA), in addition to resistance to other drugs, have emerged as a widespread cause of community infection as well. In this chapter, we describe the epidemiology and antibiotic resistance among *S. aureus* and other species with special reference to ophthalmic infections and focus on newer approaches for treatment of staphylococcus infection like phage therapy and vaccines.

2. Staphylococcus in wound and eye infections

Staphylococcus aureus, a gram-positive bacterium, discovered in 1880's has been shown to be a potential pathogen causing infections such as minor skin infections and post-operative wound infection. Since the introduction of penicillin for the treatment, the mortality rate of individuals caused by *S. aureus* infection was about 80%. After emergence of penicillin resistance and introduction of methicillin in 1961, *S. aureus* developed resistance to methicillin due to acquisition of the *mecA* gene. During last 47 years, various hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) and later virulent community-associated MRSA (CA-MRSA) clones characterised by the presence of toxin Panton-Valentine-leukocidin (PVL), were reported (Deurenberg and Stobberingh, 2008).

Staphylococci have a special relationship with the eye. On one hand, almost all species of staphylococci may be present in the lid margins or conjunctiva as normal commensals without causing disease and on the other hand, they may cause severe eye infections which may result in irreversible blindness. Colonization by resident bacteria on the ocular surface can provide a defense by inhibiting the growth of virulent bacterial strains (Iskeleli et al., 2005). However, in cases of trauma, or alteration of ocular tissue, indigenous flora may cause significant external and internal ocular infection (Speaker et al., 1991). In previous studies, native ocular flora has been shown to be predominantly *Staphylococcus* species (Iskeleli et al., 2005). While normal ocular flora has been well established in the developed world, there have been very few publications from rest of the world. In a study of the normal conjunctiva from Rajasthan, India, 86% of eyes were culture positive for bacteria and 12% positive for fungi. The most common bacterial isolates were *S. albus* (32%) followed by *S. aureus* (28%) (Tomar et al., 1971). In another study from Masungbo, Sierra Leone where analysis of conjunctival swabs obtained from healthy eyes of 276 residents showed presence of coagulase-negative staphylococci (28.6%), fungus (26.0%) and *S. aureus* (19.9%) (Capriotti et al., 2009). Many studies have not speciated the staphylococci from normal lids and conjunctiva, however, *S. epidermidis* is reported to be the most common species (McCulley et al., 1982).

Both coagulase negative and positive staphylococci are responsible for a variety of anterior and posterior segment of eye infections such as blepharitis, canaliculitis, dacryocystitis, conjunctivitis, keratitis, scleritis, endophthalmitis, preseptal and orbital cellulitis etc. Important attributes of organisms causing ocular infections include virulence, invasiveness, numbers of organism entering the host tissues and the site of entry. Coagulase, lipase and esterase are important bacterial enzymes produced by staphylococci associated with blepharitis. Several characteristics of the host also determine the effect of bacterial virulence and development of disease. Age, use of drugs and contact lens use, trauma, surgery etc. may also influence the effect of virulence factors besides presence of risk factors e.g., dry eye states, chronic nasolacrimal duct obstruction, previous ocular disease etc. Tissue injury can result from direct action of bacteria and their toxins, as well as from bacteria induced inflammation. Immunopathologic activities include recruitment of polymorphonuclear cells, macrophages and lymphocytes. Mediators of inflammation such as histamine, tumour necrosis factor, cytokines, leukotrienes, prostaglandins etc. play important role in interaction with the bacteria and their removal or proliferation.

Type of endophthalmitis	Geographic area	Duration of study	No. of patients	No, of isolates	% of CoNS	References
Posttraumatic	India	7 years	182	139	17.3	(Kunimoto et al., 1999b)
Postoperative	India	7 years	206	176	46.0	(Kunimoto et al., 1999a)
Postoperative	Singapore	5 years	34	21	57.0	(Wong and Chee, 2004)
Postoperative	India	--	80	37	62.6	(Srinivasan et al., 2002)
Postoperative	USA	5 years	278	313	49.9	(Benz et al., 2004)

Table 1. Prevalence of *Staphylococcus* species in endophthalmitis in various studies

Inflammation of the lid margin or blepharitis may be anterior or posterior, the former involving the lash line and the latter meibomian glands. Both the conditions may be associated with skin diseases such as dermatitis (seborrhoeic or atopic) and rosacea. The anterior blepharitis with lash collarettes, crusting, lid ulceration and folliculitis is usually associated with *S. aureus.* The most common form of bacterial conjunctivitis is the acute mucopurulent form of *S. aureus.* This may be associated with obstruction of the Naso-lacrimal duct. *S. aureus* conjunctivitis can become chronic due to its affinity for the eyelid margin and the resultant blepharitis. Coagulase negative staphylococci (CoNS), characteristically the endogenous flora of the ocular surface, are most of the common cause of postoperative endophthalmitis world over (Callegan et al., 2002; Kunimoto et al., 1999a, Benz et al., 2004, Wong and Chee, 2004). CoNS also rank first among bacteria causing posttraumatic endophthalmitis of which 45.3% isolates belong to gram positive cocci and 17.3% of these were belong to gram positive bacilli (Kunimoto et al., 1999b). Whereas CoNS do not commonly cause endogenous endophthalmitis, *S. aureus* have been reported from such infection (Callegan et al., 2002). **Table 1.** shows the prevalence of *Staphylococcus* species in patients with endophthalmitis.

Microbial keratitis is a serious infection of the cornea that may be caused by a variety of organisms including staphylococci. Most of the studies from developed countries such as the USA (Liesegang and Forster, 1980, Ormerod et al., 1987, Asbell and Stenson, 1982) (except southern USA) and Australia (McClellan et al., 1989) have listed *S. epidermidis* or coagulase negative staphylococci as the leading cause of bacterial keratitis. In India, the leading cause of bacterial keratitis varies; however, some investigators have listed staphylococci as the commonest bacteria (Gopinathan et al., 2009). It is possible that some investigators may have considered *S. epidermidis* or coagulase negative staphylococci as a normal commensal of the conjunctiva and underreported the isolation of these organisms from corneal samples. Few studies have recommended application of certain criteria to determine significance of a positive culture from corneal scrapings (Gopinathan et al., 2009). Since *S. epidermidis* form the commonest commensal of the extraocular surfaces, it is highly probable that these organisms invade corneal tissues compromised by antimicrobial and / or corticosteroid therapy or trauma.

For treatment of eye infections, antibiotics are usually administered topically as eye drops or intraocular injections, depending on the clinical condition. Other routes of administration such as subconjunctival injection are rarely used. Topically administered drugs have major advantage of localized drug effects, avoidance of hepatic first pass metabolism, and convenience. The disadvantage is low bioavailability to intraocular tissues, estimated to be only 1-10% (Davies, 2000). A large number of eye drops for topical therapy are available for extraocular eye infections, that include fluoroquinolones, macrolides, aminoglycosides, glycopeptides, tetracyclines, chloramphenicol, Neosporin (bacitracin, neomycin, polymyxin).

A broad range of three generations of fluoroquinolones are available such as ciprofloxacin (0.3%), ofloxacin (0.3%), levofloxacin (0.5% and 1.5%) , gatifloxacin (0.3%) and moxifloxacin (0.5%, preservative free) as eye drops also. Gatifloxacin and moxifloxacin, the newer fourth generation fluoroquinolones that target both DNA gyrase and topoisomerase IV are highly effective against gram positive bacteria including staphylococci in human and animal corneal ulcer model (Romanowski et al., 2005, Aliprandis et al., 2005). However, gatifloxacin was shown to be more effective than moxifloxacin against staphylococci (Reddy et al., 2010).

Fluoroquinolone eye drops are widely used for prophylaxis before eye surgery to prevent postoperative infection, most commonly caused by CoNS. Recently, intracameral injection of moxifloxacin has been found to be safe and effective in reducing the rate of postoperative endophthalmitis following cataract surgery (Lane et al., 2008).

Historically, the aminoglycosides have been the mainstay in the treatment of ocular infections. However, increasing resistance has limited their use in recent years in the treatment of staphylococcal infections. Glycopeptides such as vancomycin and teicoplanin the bactericidal antibiotics which inhibit peptidoglycan synthesis in the bacterial cell wall by complexing with cell wall precursors are highly effective against staphylococci including methicillin resistant staphylococci. However, eye drops are not yet available. Injectable vancomycin is routinely used for intravitreal injection (1mg/0.1ml) for the treatment of bacterial endophthalmitis. Emergence of vancomycin resistance has been reported in CoNS (Schwalbe et al., 1987). Topical ocular formulations of erythromycin are effective for conjunctivitis and blepharitis, however clarithromycin and azithromycin are derivatives that offer significant advantage over erythromycin owing to their expanded spectra (Barry et al., 1988).

Systemic infection with methicillin resistant *S. aureus* (MRSA) is known to cause morbidity and mortality. The prevalence of MRSA in ocular infections varies in different studies. While it is reported to be as low as 3% in England (Shanmuganathan et al., 2005), it is high (25-64%) in Japan (Fukuda et al., 2002). However, Indian workers have also reported increasing prevalence of MRSA over the years (Bagga et al., 2010). These authors showed decreased susceptibility to fluoroquinolones among MRSA from ocular infections. Shanmuganathan et al. (2005) found the MRSA susceptible to chloramphenicol and gentamicin and resistant to third generation fluoroquinolones (ciprofloxacin and ofloxacin) and cefazolin. Topical administration of fortified cefazolin (5%) was recommended for the treatment of staphylococcal keratitis based on in vitro susceptibility of *S. aureus* and CoNS (Sharma et al., 1999, Sharma et al., 2004). In an ongoing study, 4 of the 45 isolates (8.9%) of *S. aureus* from eye infections were MRSA (Kar et al., 2010). Using microbroth dilution and E test, a high level of resistance to fluoroquinolones but susceptibility to cefazolin, vancomycin and chloramphenicol was found among both MRSA and MSSA strains (Kar et al., 2010).

3. Antibiotic resistance

Antibiotics that are used against *Staphylococcus* spp. basically target cell wall synthesis, protein synthesis, nucleic acid synthesis and other metabolic pathways. The selection pressure applied by the antibiotics that are used in clinical and agricultural settings has promoted the evolution and spread of genes that confer resistance (Allen et al., 2010). Resistance to various antibiotics can be either internal or acquired by horizontal gene transfer via various mobile genetic elements like plasmids, transposons, integrons, etc. Internal mechanisms include mutational modification of gene targets, over expression of various efflux pumps; whereas acquired resistance involves enzymatic inactivation of the drug and bypassing of the target.

Exposure to antibiotics may lead to the formation of persister cells, small colony variants (SCVs), biofilms and over-expression of efflux pumps (Lewis, 2008, Singh et al., 2009, Proctor et al., 1998, Kwon et al., 2008, Martinez et al., 2009b) (Fig. 1). Persisters are dormant, multidrug

tolerant variants of regular cells that are formed through a combination of stochastic and deterministic events in microbial populations (Lewis, 2010). Persisters over express genes such as chromosomal toxin-antitoxin modules that shut down their cellular functions, therefore, antibiotictarget inducing dormant cell to become tolerant to the lethal action of antibiotics (Keren et al., 2004, Singh et al., 2009). Another major problem posed by persister cells is they hide at various niches evading the host immune system, such as central nervous system (*Treponema pallidum*), macrophages or granulomas (*Mycobacterium tuberculosis*), stomach (*Helicobacter pylori*), gallbladder (*Salmonella typhi*) etc. (Jayaraman, 2008).
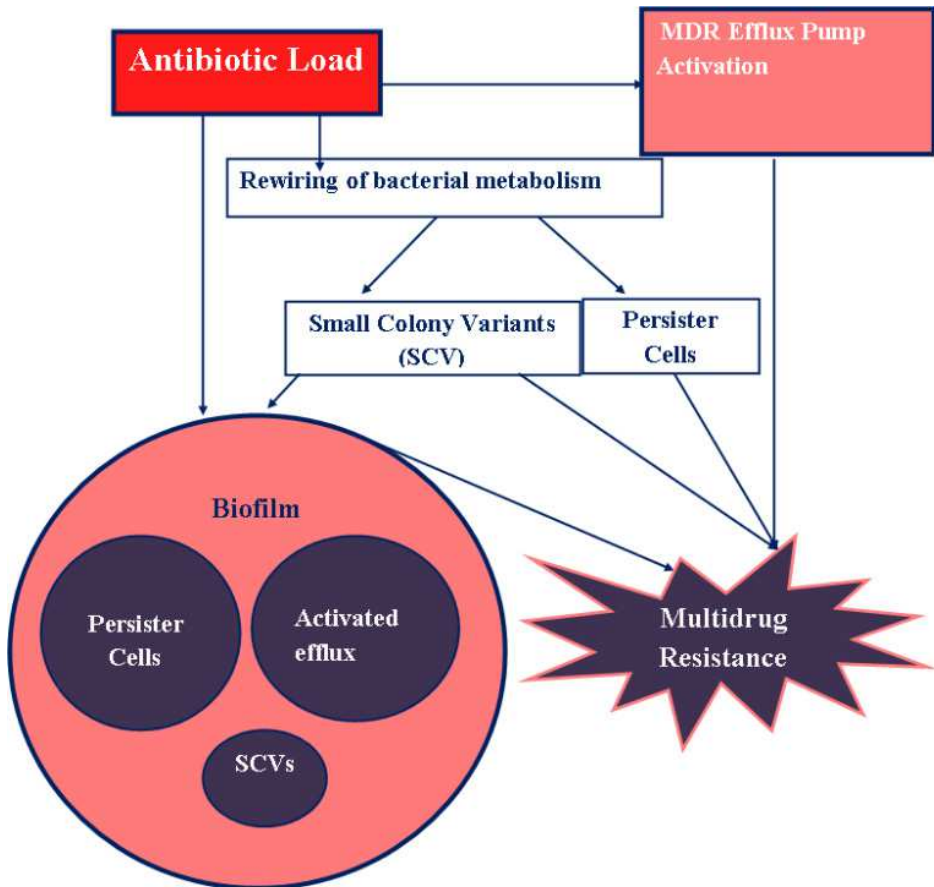
Fig. 1. Sub-inhibitory concentrations of antibiotics lead to formation of persister cells (Lewis, 2010), small colony variants (Proctor et al., 1998), biofilms (Kwon et al., 2008) and over-expression of efflux pumps (Martinez et al., 2009b). Biofilms are known to harbor cells with these kinds of modifications (Singh et al., 2010, Allegrucci and Sauer, 2007, Kvist et al., 2008). SCVs have enhanced biofilm forming capability (Singh et al., 2010). Each of these mechanisms may lead to multidrug resistance or it may be the combinatorial effect of all the above-mentioned processes.

SCVs constitute a slow-growing subpopulation of bacteria with distinctive phenotype and pathogenic traits (Proctor, 2006). They differ from the normal phenotype in their colony size, growth rate, pigmentation, haemolysis, expression of virulence factors, haemin and menadione auxotrophy, aminoglycosides and cell wall inhibitors action (Singh et al., 2009). Defective respiratory activity serves as the biochemical basis for the development of SCVs (Proctor et al., 1998). SCV of *S. epidermidis* may play a role in the pathogenesis of prosthetic valve endocarditis (Baddour and Christensen, 1987), and catheter-induced endocarditis (Baddour et al., 1988). Several findings mandate the investigation of small colony variants for persistent infections (Proctor et al., 1994, Proctor et al., 1998, Spearman et al., 1996, Kahl et al., 1998, Abele-Horn et al., 2000).

Bacteria in biofilms can tolerate ten to thousand fold higher levels of antibiotics than the genetically equivalent planktonic bacteria (Resch et al., 2005). Staphylococcal biofilms cause biomaterial-associated infections which do not respond to antimicrobial treatment often requiring removal of the same leading to substantial morbidity and mortality (Gotz and Peters, 2000). It has also been observed that biofilms harbour persister cells and small colony variants (Singh et al., 2010, Allegrucci and Sauer, 2007) (Fig. 1). Whereas planktonic persisters are eliminated by the immune system *in vivo*, persisters in biofilms serve as a shield evading the immune response (Lewis, 2010). According to Levin and Rozen (Levin and Rozen, 2006), a reservoir of such shielded persisters is a potential source for the emergence of heritable antibiotic resistance.

Kvist et al., (2008) reported the enhanced activity of efflux pumps in the bacteria residing in the biofilms (Fig. 1). The authors argued that the cramped environment in the biofilm demands better waste management leading to escalation of efflux pumps thereby increasing the antibiotic resistance of the biofilm cells. Reduction in biofilm formation was observed with the addition of efflux pump inhibitors (Kvist et al., 2008). Under physiological conditions, efflux pumps are involved in housekeeping activities like detoxification of intracellular metabolites, cell homeostasis, intracellular signal trafficking and bacterial virulence in animal and plant hosts. However, in the presence of high concentration of antibiotics and other environmental factors, they can shift their functional roles (Martinez et al., 2009a).

Antibiotics and their resistance genes were evolved in non-clinical environments in the pre-antibiotic usage era. Some antibiotics which may serve signalling purposes at the low concentration are probably found in natural ecosystems. Resistance determinants to these antibiotics were originally selected in their hosts for metabolic purposes or signal trafficking. Other antibiotic-resistance genes have been obtained by virulent bacteria through horizontal gene transfer (Martinez et al., 2009a). For example, *S. aureus mecA* gene is located on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCCmec) (Tsubakshita et al., 2010), horizontally acquired from other staphylococcal species *S. sciuri* (Couto et al., 1996) and *S. fleurettii* (Katayama et al., 2000). Also high level of vancomycin resistance is associated with carriage of *vanA* cluster encoded by Tn1546 transposon, first reported in *Enterococcus* species (Uttley et al., 1988). VRSA isolates from Michigan and Pennsylvania were found to harbor plasmids of 57.9Kb and 120Kb respectively carrying the transposon (Weigel et al., 2003, Tenover et al., 2004).

Resistance to fluoroquinolones offer a classic example of point mutations (e.g., *gyrA&B*, *grlA&B*) and efflux mediated resistance (Morar and Wright, 2010). Point mutations in

particular regions of each enzyme subunit, known as Quinolone-Resistance-Determining-Region (QRDR) makes the enzyme less susceptible to inhibition by fluoroquinolones. The level of resistance increases in a stepwise manner each time with an additional mutation in target enzyme (Hooper, 2001). Selection pressure exerted by enhanced use of quinolones have led to the emergence of resistant strains carrying mutations within the endogenous transport system that improve affinity of the efflux system for quinolones (Ohshita et al., 1990, Chopra, 1992). The quinolone resistance in *S. aureus* also involves enhanced efflux by the Nor family of multidrug efflux pumps (McCallum et al., 2010). Several reports have linked increased expression of NorA to reduced susceptibility to chloramphenicol, beta-lactams, tetracycline, puromycin and some dyes, such as ethidium bromide (McCallum et al., 2010, Hooper, 2001, Ruiz, 2003). Point mutations in *norA* gene have been associated with reduced uptake of norfloxacin by the cell (Ohshita et al., 1990). NorB and NorC are the other members of Nor family encoding fluroquinolone resistance (Truong-Bolduc et al., 2006).

Although fluoroquinolones are considered first-line treatment of ocular infections, 85% of MRSA are resistant to ophthalmic fluoroquinolones (McDonald and Blondeau, 2010). This rise in resistance mandates the need for new agents. Basifloxacin, a novel fluoroquinolone was approved as a topical agent for treatment of bacterial conjunctivitis in May 2009 demonstrated rapid bactericidal activity against isolates that showed in vitro resistance to other fluoroquinolones, beta-lactams, macrolides and aminoglycosides (Haas et al., 2010). Moreover, basifloxacin lack systemic counterpart, thereby eliminating the contribution of systemic use of this drug to the emergence of resistance, although cross resistance from other systemic fluoroquinolones is possible (McDonald and Blondeau, 2010).

The direct relationship between the development of Linezolid (the last-line agent) resistance and prolonged exposure of the drug among cystic fibrosis patients was reported by Endimiani et al., (2011). Linezolid resistance in *S. aureus* is uncommon though there are reports of mutations in 23S rRNA and ribosomal protein L3 and L4 encoded by *rplD* gene and *rplC* genes (Locke et al., 2009). However, the most worrisome mechanism involving acquisition of the methytransferase *cfr* that methylates the 23S rRNA associated with mobile genetic elements had first been identified in 16.5 kb multi-drug resistance plasmid in *Staphylococcus sciuri* (Kehrenberg et al., 2005).

4. Therapeutics & therapy

With the ample evidence of strong association between antibiotic resistance and antibiotic consumption the scientific community should also come up with alternative means of antibacterial therapies, besides legitimately using available antibiotics, which can be used either alone or in conjunction with the antibiotics. Here we discuss some of the alternative strategies including vaccine development, phage therapy, use of lytic enzymes and plant-derived antibacterials. There are some reports on the use of nanoparticles as an efficient means of delivering antibacterials.

4.1 Staphylococcal vaccines

S. aureus has devised various mechanisms to evade the immune system. (i) two immunoglobulin binding proteins (protein A and Sbi), (ii) immune cell lysing toxins (Hlg, PVL), (iii) proteins interfering with complement activation (SCIN– staphylococcal

complement inhibitor) and (iv) chemotaxis of neutrophils inhibiting peptides. Production of superantigens by *S. aureus* leads to allergy and immunosuppression. *S. epidermidis* relies primarily on cell-surface polymers and the ability to form a biofilm to survive in the host (Foster, 2005). However, protective role of antistaphylococcal antibodies from staphylococcal infection has been well documented in literature (Dryla et al., 2005). Holtfreter and Broker (2005) reported that carriers have high titers of neutralizing antibodies specific for those superantigens that are expressed by their colonizing strain. This carriage status confers strain specific humoral immunity, which may contribute to protection during *S. aureus* septicemia. Substantial controversy exists as to whether staphylococcal infections may be prevented by vaccination and, if so, which antigens should be selected and patients targeted for vaccination. In a comprehensive review on immune-therapeutics for staphylococcal infections by Ohlsen and Lorenz (2010) described the necessity for the development of both passive and active immunotherapies against *Staphylococcus*. The underlying criteria for the selection of targets e.g. gene products or toxins, should be conservability and expression in most of the clinical isolates.

4.1.1 MSCRAMM (Microbial Surface Component Recognizing Adhesive Matrix Molecules)

Some of the surface proteins of *S. aureus* have been exploited for immunotherapy. MSCRAMM protein family represents prototype of targets because of their exposed location and virulence involvement (Ohlsen and Lorenz, 2010, Flock, 1999). The best characterized MSCRAMM proteins include (i) clumping factor B (clfB), (ii) collagen-binding protein (Cna), and (ii) fibronectin-binding protein (FnBPA) (Ohlsen and Lorenz, 2010, Garcia-Lara et al., 2005). Veronate (Inhibitex) was developed by including anti-clfA and SdrG (*S. epidermidis* protein) from selected human donors (Patti, 2005), but failed to reach its target endpoints for the protection because of low birth weight babies at clinical trial III (Ohlsen and Lorenz, 2010).

Aurexis® is a humanized monoclonal antibody that recognizes clumping factor A (ClfA), a cell surface protein expressed by virtually all strains of *S. aureus*. Aurexis® binds with high affinity and specificity and interferes with *S. aureus* ability to colonize and spread to fibrinogen containing substrates such as wound sites, biomaterial coated implants, and damaged endovascular tissues. Inhibitex is actively seeking a corporate partner(s) for the continued clinical development of Aurexis® (http://www.inhibitex.com/Pipeline/Partnerships.html). Antibodies against clumping factor B (clfB) (Schaffer et al., 2006), Cna (Mamo et al., 2000), FnBPA (Zhou et al., 2006) have shown promising results. However, antibodies against against these targets have not yet been included in clinical trials (Ohlsen and Lorenz, 2010). Stranger-Jones et al., (2006) reported that the combination of four surface proteins IsdA, IsdB, SdrB, and SdrE afforded high level of protection against invasive disease or lethal challenge with human clinical *S. aureus* isolates.

4.1.2 Capsule

Capsular polysaccharides (CPs) represent the best established targets for vaccine-induced immunity to bacterial cells. About 70%–80% of *S. aureus* strains produce one of two CP antigens e.g. CP5 or CP8 (Skurnik et al., 2010). Nabi pharmaceuticals developed a vaccine StaphVax™ conjugating CP5 and CP8 to detoxified *Pseudomonas aeruginosa* exoprotein A, that failed to protect haemodialysis patients against *S. aureus* infections (Ohlsen and Lorenz, 2010,

Shinefield et al., 2002). To enhance the efficacy of StaphVax™, Pentastaph™ -pentavalent *S. aureus* vaccine was developed that included surface polysaccharide component 336, PVL and alpha-toxin to eliminate *S. aureus* by phagocytosis and neutralizing bacterial toxins. This vaccine has been evaluated in Phase II for safety and immunogenicity (Ohlsen and Lorenz, 2010).

4.1.3 Biofilm as the vaccine target

Although the significance of biofilm in infections has been recognized, there has not been much effort to develop vaccine targeting biofilms. The possible target sites for vaccine development may be the bacterial cells within the biofilm and/or biofilm matrix (Harro et al., 2010). Cerca et al., (2007) reported that an antibody developed against *Staphylococcus* Poly-N-acetyl glucosamine (PNAG) was found effective against different strains of *E. coli*. In another report, effectiveness of PNAG as vaccine candidate in *S. aureus* mediated skin abcesses and lethal *E. coli* peritonitis was demonstrated (Gening et al., 2010). However, PNAG may not be an ideal vaccine candidate against those strains possessing *icaADBC* locus because it is not produced in all biofilm-producing staphylococcal strains (Harro et al., 2010, Rohde et al., 2001). Therefore, it was suggested that vaccine studies should be focussed on the cell embedded in the matrix rather than the matrix. A proteomic approach of looking into the comparative proteomes of the planktonic and biofilm cells may be an interesting area to start with (Harro et al., 2010).

4.1.4 Whole cell vaccine

Vaccine Research International (http://www.vri.org.uk/) is developing a vaccine (SA75) using chloroform killed whole cells of *S. aureus*. Phase I clinical trials have been successfully completed. The whole cell preparation could provide a broad spectrum of *S. aureus* antigens in a single vaccine some of which had immune-stimulatory affect and act as an adjuvant generating higher antibody response against protective antigens. However, the mechanism of action is unknown and down-regulation of immune pathways by components of the vaccine cannot be ruled out (Ohlsen and Lorenz, 2010).

4.1.5 Staphylococcal enterotoxin as a vaccine candidate

Virulence factor-specific antibodies derived from vaccination or employed as therapeutics represent a potential defense against bacterial diseases (Larkin et al., 2010). Staphylococcal enterotoxins are considered potential biowarfare agents that can be spread through ingestion or inhalation (Drozdowski et al., 2010). Staphylococcal enterotoxins (SEs) and related toxic shock syndrome toxin-1 (TSST-1) act as superantigens. These protein toxins can cause acute gastroenteritis and toxic shock syndrome. There are more than twenty different SEs described to date with varying amino acid sequences, common conformations, and similar biological effects. Picomolar concentrations of these superantigenic toxins activate specific T-cell subsets after binding to major histocompatibility complex class II. Activated T-cells vigorously proliferate and release proinflammatory cytokines plus chemokines that can elicit fever, hypotension, and other ailments which include a potentially lethal shock (Larkin et al., 2009, Varshney et al., 2010).

Studies on the protective effect of non-toxic mutant GST–mTSST-1 fusion protein against staphylococcal infection, was purified and tested. Mice were immunized with the GST–mTSST-1 plus alum adjuvant and challenged with viable *S. aureus*. The results indicate the efficacy of this protein in the elimination of bacterial load from the organs as well as in the inhibition of production of pro-inflammatory cytokines due to TSST-1 in the splenic cells. Furthermore immunization with GST–mTSST-1 strongly induced the production of TSST-1 specific antibodies, especially immunoglobulin G1 and immunoglobulin G2b (Cui et al., 2005).

Varshney et al., 2010 showed that four murine monoclonal antibodies bind to conformational epitopes that are destroyed by deletion of the distal C-terminal 11 Amino acids (Varshney et al., 2010). This study, for the first time, showed that MRSA derived SEB (staphylococcal enterotoxin B) contains a deletion in the C-terminal, which affects binding of certain protective Abs. This study also demonstrated enhanced protection against SEBILS (SEB induced Lethal Shock) when two non-protective mAbs were combinedly administered *in vivo*.

Drozdowski et al., (2010) generated high-affinity SEB-specific antibodies capable of neutralizing SEB *in* vitro as well as *in vivo* in a mouse model. They described for the first time recombinantly-derived human monoclonal antibodies against SEB that possess high affinity, target specificity, and therapeutic potential for superantigen-induced toxic shock. These antibodies prevent intoxication by interfering with toxin binding to MHC II and/or TCR. In addition to potential applications for treating toxic shock syndrome, human monoclonal antibodies recognizing SEB or other bacterial superantigens may be useful if employed as an adjunct therapy with antibiotics in treating *S. aureus* infections.

STEBvax is a new vaccine developed against toxic shock syndrome. It is currently under clinical trial phase I. The vaccine is being tested for prophylactic and therapeutic use (http://clinicaltrials.gov/ct2/show/NCT00974935) .

Larkin et al., (2010) selected human monoclonal antibodies from a phage display library, using a recombinant SEB vaccine (STEBVax) incorporating site-specific mutations that prevent MHC II interactions. This group discovered that some antibody clones cross-react with SEC1, SEC2, and streptococcal pyrogenic exotoxin C (SpeC), while others were highly specific for SEB. Many of the antibodies effectively inhibited T-cell activation by SEB in vitro, bound to toxin with nanomolar affinity, and prevented SEB-induced toxic shock in vivo. This recombinantly-derived, human monoclonal antibodies against SEB had high affinity, target specificity, and therapeutic potential for superantigen-induced toxic shock. These antibodies prevented intoxication by interfering with toxin binding to MHC II and/or T-cell antigen receptors. The author suggested the potential applications for treating toxic shock syndrome, human monoclonal antibodies recognizing SEB or other bacterial superantigens may be useful if employed as an adjunct therapy along with antibiotics in treating difficult *S. aureus* infections (Larkin et al., 2010).

4.1.6 Future perspectives in vaccine development

As discussed earlier, comparative proteomic analysis of planktonic and biofilm cells is an interesting area to look for vaccines against biofilm. Another important strategy is to look for immunodominant antigens. This strategy has led to the identification of a wide range of surface and extracellular target antigens such as IsdB, GrfA, IsaA, IsaB, Atl, IsdA, IsdH,

FmtB, SspA, SspB and Lip (Lorenz et al., 2000, Etz et al., 2002, Clarke and Foster, 2006). The rationale behind such a strategy is that the patients may develop antibodies against specific staphylococcal antigens during infection that may be critical for combating the infections (Ohlsen and Lorenz, 2010).

Another promising strategy is the reduction of nasal colonization by *S. aureus* as several studies have shown that nasal carriers have increased risk of developing infections by endogenous strains (von Eiff et al., 2001, Wertheim et al., 2004). Vaccination with clumping factor B, iron-responsive surface determinants (Isd) A and H, teichoic acid and capsular polysaccharides have been reported to reduce the nasal colonization with *S. aureus* (Dryla et al., 2005, Clarke and Foster, 2006).

4.2 Phage therapy

The rise of multidrug resistant bacteria has enforced the resurgence of phage therapy in the West, though this mode of therapy is being practiced for several years in Eastern Europe. Some of the success stories on phage therapy are described here. The Eliava Institute in Tbilisi, Republic of Georgia, has developed a highly virulent, monoclonal staphylococcal bacteriophage active against 80-95% of *S. aureus* strains including MRSA. This product was used for local and generalized infections, including neonatal sepsis, osteomyelitis, wound infections, pneumonia etc. (Hanlon, 2007). There are some polyvalent obligate lytic *S. aureus* phages e.g. phage phi812, phageK and phage44AHJD which have been successfully tested for their efficacy in killing *S. aureus* including MRSA strains (Mann, 2008). Evaluation of phageK showed marked reduction of pathogenic and antibiotic resistant coagulase positive and negative staphylococci associated with bovine and human infections that included *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. chromogenes*, *S. capitis*, *S. hominis*, *S. haemolyticus*, *S. caprae*, and *S. hyicus*. The modified phage generated by passing through less susceptible target strain can be used in combination with phageK to increase the host range. This study had also shown the potential of delivering the phage in the form of handwash or antistaphylococcal cream (O'Flaherty et al., 2005b). Merabishvili and colleagues (2009), demonstrated laboratory-based production and quality control of a cocktail, currently under evaluation, consisting of exclusively lytic bacteriophages for the treatment of *Pseudomonas aeruginosa* and *S. aureus* infections of burn wound.

Curtin and Donlan (2006), reported use of phage *e.g.* phage456, in reducing the biofilm formation and adherence of *S. epidermidis* biofilms on both hydrogel-coated and serum/hydrogel coated silicone catheters. The presence of divalent cations in the growth medium (Mg^{++}, Ca^{++}) further increased the efficacy of phage456 in reducing biofilm formation. Polyvalent *Staphylococcus* phage combined with highly efficient *Pseudomonas* T7-like phage (phage phiIBB-PF7A) effectively showed reduction in dual species biofilms, killing and finally removal of bacteria from the host substratum (Sillankorva et al., 2010).

There were efforts to engineer bacteriophage by over-expressing proteins to target gene networks, particularly non-essential genes, to enhance bacterial killing by antibiotics. Using this approach, Lu and Collins (2007), engineered a T7 phage which significantly reduced *Escherichia coli* biofilm. They claim that this combinatorial approach may reduce the incidence of antibiotic resistance and enhance bacterial killing.

There are many advantages of using phage in therapeutics. (i) Dysbiosis can be avoided due to their specificity, (ii) Multiple administrations are not required because phage replicates at the site of infection, (iii) Phage could select resistant mutants of the selected bacteria, and (iv) Selection of new phages is rapid compared to the development of new antibiotic which may take several years. However, the disadvantage is that the causal organism needs to be identified before administering the phage (Sulakvelidze et al., 2001). Moreover, prior to the extensive therapeutic use of phages it is prudent to ensure the safety of therapeutic phages. The phages should not carry out generalized transduction and possess gene sequences having significant homology with known antibiotic resistances, phage-encoded toxins and other bacterial virulence factors (Sulakvelidze et al., 2001).

4.3 Antistaphylococcal lytic enzymes

Antistaphylococcal lytic enzymes can be broadly divided into two groups: (i) Staphylococcal phage lysins (endolysins) and (ii) Bacteriocins (e.g. Lysostaphin) (Borysowski and Gorski, 2009a).

4.3.1 Lysins

Lysins are enzymes, consist of N-terminal catalytic domain and C-terminal bacterial cell-wall binding domain, and are produced by bacteriophage that digests the bacterial cell wall (Fischetti, 2010, Borysowski and Gorski, 2009a). LysK, highly specific for the genus *Staphylococcus*, was obtained from phageK, has been effectively used in the treatment of staphylococcal infections (O'Flaherty et al., 2005a). Interestingly MV-L derived from bacteriophage phiMRII was found specific to *S. aureus* and *S. simulans* infection (Rashel et al., 2007). This phage also acted synergistically with glycopeptide antibiotics against VISA and MRSA. Moreover, MV-L induced antibodies could not abolish the bacteriolytic activity. Endolysin from another phi11 showed elimination of *S. aureus* NCTC8325 biofilm but not of *S. epidermidis* O-47 biofilm (Sass and Bierbaum, 2007). Purified endolysin (MW 53.3kDa) from virulent *S. aureus* bacteriophage Twort, *plyTW*, demonstrated cleavage of staphylococcal peptidoglycan. Upstream of *plyTW* there is Twort holin gene, *holTW*, which produces unspecific holes in the bacterial cytoplasmic membrane, degrade staphylococcal peptidoglycan through hydrolysis of alanine amino bonds (Loessner et al., 1998). CHAP (cysteine-histidine dependent amidohydrolase/ peptidase) exhibits lytic activity against staphylococcal isolates including MRSA, was identified by deletion analysis of LysK domain. CHAP can be used as single domain for therapeutic purposes over the whole enzyme as this may lower the risk of immunogenic response (Horgan et al., 2009).

4.3.2 Synergistic effect of lysins

Manoharadas et al., (2009) constructed a chimeric endolysin (P16-17) consisting of N-terminal D-alanyl-glycyl endopeptidase domain and C-terminal P16 endolysin domain and P17 minor coat protein, targeting cell wall of *S. aureus* phage. This domain swapping approach and subsequent purification resulted in finding soluble P16-17 protein, which exhibited antimicrobial activity against *S. aureus*. This protein further augmented the antimicrobial efficacy of gentamicin suggesting synergistic effect in reducing effective dose of aminoglycosides. Synergistic effect of nisin and LysH5, the endolysin encoded by phi-SauS-IPLA88 was demonstrated (Garcia et al., 2010). It was suggested that better lytic

enzymes can be constructed by using methods like protein engineering, domain swapping and gene shuffling. Owing to the non-existence of bacterial resistance to lysins someday phage lytic enzymes could be an essential strategy to combat pathogenic bacteria (Fischetti et al., 2010).

4.3.3 Lysostaphin as a therapeutic agent

Lysostaphin, a 25kDa protein possessing two functional domains: N-terminal catalytic domain and C-terminal cell wall binding domain B, is a plasmid-encoded extracellular enzyme produced by *S. simulans* biovar *staphylolyticus* (Borysowski and Gorski, 2009b). It is a Zn-containing endopeptidase that specifically cleaves the bonds between the glycine residues in the interpeptide cross-bridges of the staphylococcal peptidoglycan resulting in the hypotonic lysis of the bacterial cell (Kumar, 2008). There are several reports of lysostaphin mediated lysis of clinically relevant antibiotic resistant staphylococcal strains. Lysostaphin which is readily absorbed onto catheter surfaces without losing lytic property shows promise in the prevention of catheter-related bloodstream infections caused by CoNS and *S. aureus* (Shah et al., 2004, Borysowski and Gorski, 2009a). Lysostaphin has successfully eradicated nasal colonization of MSSA, MRSA and mupirocin-resistant *S. aureus* in experimental cotton rats (Kokai-Kun et al., 2003). In combination with lysostaphin, oxacillin or vancomycin, showed increased efficacy against MRSA (Kokai-Kun et al., 2007, Patron et al., 1999). Several workers demonstrated the effectiveness of lysostaphin in the treatment of biofilms formed by *S. aureus* and *S. epidermidis* and disruption of biofilms on glass and plastic surfaces (King et al., 1980, Walencka et al., 2005, Wu et al., 2003). Evaluation of PEGylation potential in improving lysostaphin pharmacokinetics showed substantial increase in serum drug half-life and reduced binding to anti-lysostaphin antibodies while maintaining the enzyme's lytic activity (Walsh et al., 2003).

4.3.4 Lysostaphin therapy in ocular infections

Lysostaphin was also tested as a potential means of treating some ocular infections, especially endophthalmitis and keratitis. Dajcs et al., (2001) demonstrated the effect of lysostaphin on MRSA endophthalmitis in the rabbit model. Lysostaphin when administered twice after 8h and 24h post-infection showed 88% and 50% sterilization compared to 0% sterilization in untreated controls. However, the severity of ocular inflammation could be controlled only on 8h post-infection treatment models. Lysostaphin as a probable immunizing agent was also investigated. In this study, rabbits were immunized with lysostaphin by subcutaneous, intranasal or intraocular route that showed successful retention of bactericidal activity in vivo, in spite of the high titre of anti-lysostaphin antibodies (Dajcs et al., 2002, Balzli et al., 2010).

4.3.5 Synergistic effects

Synergistic inhibition by ranalexin (a cationic peptide) in combination with lysostaphin resulted in an enhanced bactericidal effect. This finding, therefore, suggested that dressings could be impregnated with ranalexin and lysostaphin to treat wound infections caused by MRSA (Graham and Coote, 2007, Desbois et al., 2010). Furthermore lysozyme has been reported enhancing lysostaphin activity (Cisani et al., 1982). Combinatorial action of various

beta-lactam antibiotics or mupirocin or gentamicin enhancing lysostaphin activity was reported by various groups (Polak et al., 1993, Climo et al., 2001, Kiri et al., 2002, LaPlante, 2007). Thus, it is concluded that lysostaphin is an effective agent as pre- and post-treatment option for staphylococcal infections though CoNS showed generally weaker effect than *S. aureus* (Borysowski and Gorski, 2009a). This was because of presence of higher amount of serine than glycine in peptidoglycan of coagulase-negative staphylococci (Kumar, 2008).

4.4 Plant-derived antibacterials

Plant-derived antibacterials are of three types: (i) traditional antibiotics, (ii) antibacterials that target bacterial virulence, and (iii) inhibitors of MDR pump. The first two categories have not been explored in detail (Lewis and Ausubel, 2006). The details of third category of compounds obtained from plant sources, their properties as MDR/ EPI inhibitors and its use as antibacterial against staphylococcal infection are summarized in Table 2.

Identification of EPIs from natural sources is still in infancy. However, the chemical diversity of plants and microorganisms and their requirement for nutrients to synthesize such compounds should make the search for EPIs from such sources an attractive option (Stavri et al., 2007).

4.5 Nature's backyard

Resistance of microbial pathogens to antibiotics is a serious threat to the well-being of mankind. Recently, two small molecules, platensimycin, identified from strain of *Streptomyces platensis* isolated from soil sample in South Africa, by using antisense differential sensitivity whole-cell screening program, targeting the fatty acid biosynthesis pathway of gram-positive bacteria. The platensimycin ($C_{24}H_{27}NO_7$, MW 441.47) comprises of two distinct structural element connected by an amide bond, is active against MRSA, VISA, Vancomycin-resistant enterococci and linezolid and macrolide resistant pathogens (Wang et al., 2006) (Fig. 2A).

Continued screening led to the discovery of platencin ($C_{24}H_{27}NO_6$, MW 425.2), a novel product that is chemically and biologically related to platensimycin which exhibits broad-spectrum antibacterial activity against gram positive bacteria which inhibit fatty acid biosynthesis (Fig. 2B). These molecule targets two essential proteins, beta-ketoacyl synthase II (FabF) and III (FabH) (Wang et al., 2007). These studies reflect upon the fact that nature holds the treasure trove of antibiotics which are yet to be explored.
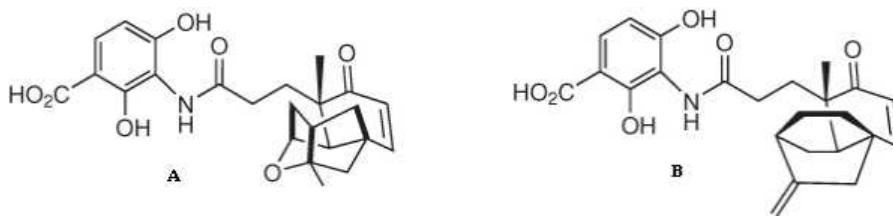

Fig. 2. Chemical structure of Platensimycin (A) and Platencin (B).

	Common name & plant species name	Compound	Properties	Total effect	Synergistic effect	References
1	Barberry & *Berberis* species	Berberine & 5'-methoxyhydnocarpin	Hydrophobic cation increases membrane permeability and intercalate DNA	Inhibit MDR	Antibacterial	(Stermitz et al., 2000)
2	Golden seal & *Hydrastis canadensis*	Berberine & 5'-methoxyhydnocarpin	5'-methoxyhydnocarpin-linking of berberine to INF_{55}	Inhibit MDR	Antibacterial	(Ball et al., 2006)
3	Silvery lupine & *Lupinus argenteus*	Isoflavones	Enhances activity of berberine and norfloxacin	Inhibit MDR	Antibacterial	(Morel et al., 2003)
4	Fabaceae & *Dalea versicolor*	Phenolic metabolites	Enhances activity of berberine, erythromycin and tetracycline	Inhibit MDR	Antibacterial	(Belofsky et al., 2004)]
5	Smoke tree & *Dalea spinosa*	2-arylbenzofuran aldehyde & Phenolic compounds; SpinosanA, Pterocarpan & Isoflavone	Enhances activity of berberine	Inhibit MDR	Antibacterial	(Belofsky et al., 2006)
6	Tea	Epicatechin gallate & Epigallocatechin gallate	Enhances activity of norfloxacin and tetracycline	Inhibit MDR	Antibacterial	(Gibbons et al., 2004, Sudano Roccaro et al., 2004)
7	Rosemery & *Rosmarinus officinalis*	Diterpines, Carnosic acid & Carnesol	Potentiate activity of tetracycline and erythromycin	Inhibit MDR	Antibacterial	(Oluwatuyi et al., 2004)
8	Gipsywort & *Lycopus europaeus*	Lipophilic extract	Potentiate activity of tetracycline and erythromycin	Inhibit MDR	Antibacterial	(Gibbons et al., 2003)
9	Grapefruit oil & *Citrus paradisi*	Coumarin derivative: Bergamottin epoxide & Coumarin epoxide	Enhances activity of ethidium bromide and norfloxacin	Inhibit MDR	Antibacterial	(Abulrob et al., 2004)
10	Piperine & *Piper nigrum* & *Piper longum*	Piperine	Potentiating action of piperine in combination with ciprofloxacin	Inhibit MDR	Antibacterial	(Khan et al., 2006)

Table 2. Properties of MDR/ EPI inhibitors isolated from medicinal plants and its use as antibacterial agent against Staphylococcal infection.

5. Conclusions

Extensive applied and basic research is needed to come up with strategies combating the major challenges of staphylococcal infections. Recently, folic acid tagged chitosan nanoparticles were effectively used to deliver vancomycin. It proved to be an efficient method to increase the bioavailability of the same (Chakraborty et al., 2010). Researchers have developed Nitric oxide releasing nanoparticles as treatment for skin and soft tissue infections successfully tested in murine models (Han et al., 2009, Englander and Friedman, 2010).

Use of vaccines and phages for the treatment and control staphylococcal infections might be a sustainable alternative to antibiotics. The advent of high throughput sequencing has led to the analysis of phage genomes and better understanding of phage evolution, phage-host interaction, bacterial pathogenicity, phage ecology and origin of phages (O'Flaherty et al., 2009). With the background knowledge of phage genomics, it will be possible to approach phage therapeutics cautiously and effectively. Future research should focus on multidisciplinary approach on the development of alternative/conjunctive strategies for treatment and prevention of staphylococcal infections.

6. Acknowledgement

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Antibiotic-resistant bacterial strains remain a major global threat, despite the prevention, diagnosis and antibiotherapy, which have improved considerably. In this thematic issue, the scientists present their results of accomplished studies, in order to provide an updated overview of scientific information and also, to exchange views on new strategies for interventions in antibiotic-resistant bacterial strains cases and outbreaks. As a consequence, the recently developed techniques in this field will contribute to a considerable progress in medical research.

How to reference

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