

## Alternative therapies in *Staphylococcus aureus* diseases

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***Staphylococcus aureus* is a common pathogen responsible for health-care-associated infections as well as community acquired ones. It is the etiological factor of a wide spectrum of infections. Therapeutic problems are caused by resistance of *S. aureus* to many antibiotics, specifically to methicillin (methicillin-resistant *S. aureus*, MRSA). In such cases a limited spectrum of antibiotics may be used and prolonged hospitalization is costly. Hence, there is an urgent need for the development of alternative antibiotic therapeutics. This work reviews the current knowledge concerning prospective treatment of staphylococcal diseases.**

**Key words:** *Staphylococcus aureus* diseases, alternative therapies

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### BACKGROUND

*Staphylococcus aureus* is a pathogen able to bypass all barriers of the host defence system as it possesses a wide spectrum of virulence factors (Feng *et al.*, 2008; Plata *et al.*, 2009). Despite the increasing knowledge on this subject it is still difficult to prevent or effectively treat staphylococcal infections in many cases.

Therapeutic problems are caused by infections with strains which are resistant to many antibiotics and specifically to methicillin (methicillin-resistant *S. aureus*, MRSA) (Ito *et al.*, 2001; Ito & Hiramatsu, 2003). Due to the wide presence of infections caused by multiresistant strains the development of new therapeutic strategies - including antibiotic and alternative therapies as well as prophylaxis strategies, which are important for patients before invasive medical procedures — is a busy field of research (Grinholc *et al.*, 2008b; Hiramatsu *et al.*, 1997; Kampf *et al.*, 2003; Kaiser *et al.*, 2004; Rayner & Munchhof, 2005; Kowalski *et al.*, 2005; Trampuz & Zimmerli, 2006; Livermore, 2006; Tacconelli, 2006; Karchmer, 2006; Finch, 2006; Goldstein, 2007; Witte *et al.*, 2007).

It should be noted that problems associated with the HA-MRSA (healthcare associated MRSA) infections are related not only to their resistance to beta-lactam antibiotics but also to their lack of sensitivity towards other antibacterial drugs. According to many authors resistance among HA-MRSA to fluoroquinolones ranges from 75% to 100% of strains, gentamicin 59% — 100%, clindamycin 60% — 100%, erythromycin 71% — 100%, and trimetoprim/sulfamethoxazole 30% — 97% of strains (Sola *et al.*, 2002; Savas *et al.*, 2005; Conceicao *et al.*, 2007; Kuint *et al.*, 2007; Huang *et al.*, 2007; Kurlenda *et al.*, 2007; Gonlugur *et al.*, 2003).

As a result of the increasing percentage of infections caused by the MRSA displaying intermediate sensitivity or resistance to vancomycin, antibiotic therapy is becoming more difficult and often fails (Rayner & Munchhof, 2005; Levy, 2005; Trampuz & Zimmerli, 2006; Livermore, 2006; Karchmer, 2006; Finch, 2006). According to the first report in 1997 regarding the therapeutic failure of vancomycin in the treatment of infection caused by MRSA with reduced sensitivity to this antibiotic, the development of resistance towards glycopeptides, which are an important group of antibacterial drugs against multidrug resistant (MDR) HA-MRSA has become a threat (Hiramatsu *et al.*, 1997). This is the reason for the continuing research regarding developing therapy methods alternative to the use of antibiotics. This work is an overview of the current state of knowledge based on literature from recent years and also presents the Authors' point of view.

### STAPHYLOCOCCUS AUREUS DISEASES

*S. aureus* is a cause of local infections which can in later stages develop into systemic infections such as bacteremia and sepsis both in adults (Gottlieb *et al.*, 2000; Priest & Peacock, Jr., 2005; Whyte *et al.*, 2005; Collins, 2007; Benfield *et al.*, 2007; Desachy *et al.*, 2007) and in children (Healy *et al.*, 2004; Regev-Yochay *et al.*, 2005; Kuint *et al.*, 2007). The second group of infections caused by *S. aureus* are toxin-mediated diseases (Ladhani & Garbush, 2005; Murray, 2005; Vayalumkal & Jadavji, 2006; Chi *et al.*, 2006; Kurlenda *et al.*, 2009).

Local infections are associated with skin and soft tissue damage such as wound infections (Kalmeijer *et al.*, 2000), skin infections (folliculitis, furuncles), cellulitis, abscesses (Ladhani & Garbush, 2005; Vayalumkal & Jadavji, 2006; Moran *et al.*, 2006; Chi *et al.*, 2006) and deep infections such as myositis (Roberts & Chambers 2005), osteomyelitis (Smeltzer & Gillaspay, 2000; Lew & Waldvogel, 2004; Priest & Peacock, Jr., 2005; Davis, 2005; Dzwonkowska *et al.*, 2007), pericarditis (James, 2001; Browatzki *et al.*, 2006), endocarditis (Gottlieb *et al.*, 2000; Fowler, Jr., *et al.*, 2005b; Chesi *et al.*, 2006), septic arthritis (Davis, 2005), and pneumonia (Le *et al.*, 2001; Francis *et al.*, 2005). The device-related infections can be caused by a foreign body such as intravascular catheters (Priest & Peacock, Jr., 2005; Collins & Hampton, 2005; Fowler, Jr. *et al.*, 2005a; Nowakowska *et al.*, 2007), propylene nets (Abele-Horn *et al.*, 2000), ventriculoperitoneal shunts

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**Abbreviations:** MDR, multidrug resistant; MSSA, ?; MRSA, methicillin-resistant *S. aureus*; HA-MRSA, healthcare associated MRSA; SFD, Staphylococcal Foodborne Diseases SSSS, Staphylococcal Scalded Skin Syndrome; TSS, Toxic Shock Syndrome

**Table 1. *Staphylococcus aureus* virulence factors**

| Group of virulence factors                                    | Selected factors  | Genes  | Clinical symptoms  |
|---|---|--|--|
| Connected with host tissue adhesion                           | MSCRAMMs (e.g. clumping factor, fibronectin-, collagen- or sialoprotein-binding proteins)   | <i>clfA, clfB, fnbA, fnbB, cna, sdr, bbp</i>       | Endocarditis, osteomyelitis, endoprosthesis related infections |
| Responsible for <i>S. aureus</i> persistence in host organism | Biofilm (polysaccharide production responsible for intercellular adhesion), small colony variant, <i>S. aureus</i> ability to persist in host cells | locus <i>ica, hemB</i>                             | Chronic infections   |
| Responsible for avoiding/fighting host immune response        | Leukocidins (e.g. Pantone-Valentine leukocidin and toxin $\gamma$ ), capsular polysaccharides, protein A, extracellular matrix binding protein      | <i>lukS-PV, lukF-PV, hlg, cap5, cap8, spa, eap</i> | Invasive skin infections, pneumonia, abscesses                 |
| Connected with host tissue penetration                        | Proteases, lipases, nucleases, hylaruronidases, phospholipase C, metalloproteases   | <i>V8, hysA, hla, plc, sepA</i>                    | Tissue lesions   |

(Spanu *et al.*, 2005), pacemakers (Seifert *et al.*, 2003) and different kinds of implants used in orthopedics (Hudson *et al.*, 1999; Jarraud *et al.*, 2002; Lew & Waldvogel, 2004; Davis, 2005). Both device-related diseases and local infections require simultaneous occurrence of many factors enabling colonization and invasion of a host tissue (Table 1).

Toxins are the cause of toxin-mediated diseases such as Toxic Shock Syndrome (TSS) (Chi *et al.*, 2006; Murray 2005), Bullous impetigo, Staphylococcal Scalded Skin Syndrome (SSSS) (Chi *et al.*, 2006; Gravet *et al.*, 2001; Ladhani & Garbash, 2005; Vayalumkal & Jadavji, 2006) and Staphylococcal Foodborne Diseases (SFD) (Murray 2005; Omoe *et al.*, 2002; Nema *et al.*, 2007; Naffa *et al.*, 2006; Sandhu & Kanwar, 2004) (Table 2).

## CURRENT ANTIBIOTIC OPTIONS FOR TREATMENT OF *S. AUREUS* INFECTIONS

Treatment of MSSA infections is not challenging as wide range of antibiotics can be used, especially  $\beta$ -lactam ones (including penicillins with  $\beta$ -lactamase inhibitors, e.g. amoxicillin/clavulanic acid) or narrow-spectrum izoxazolil penicillins resistant to staphylococcal  $\beta$ -lactamases (cloxacillin). Moreover, MSSA strains seem to be susceptible to other groups of antibiotics, aminoglycosides (gentamycin, amikacin), fluorochinolones (ciprofloxacin, levofloxacin), lincosamides (clindamycin), macrolides (erythromycin), tetracyclines (doxycycline, minocycline) and glycopeptides (vancomycin, teicoplanin) (Gilbert *et al.*, 2011). In the case of community acquired MRSA (CA-MRSA) infections, due to the resistance mechanism,  $\beta$ -lactam antibiotics cannot be used, but the susceptibility of these strains to Rother groups of antibiotics seem to be preserved (DeLeo *et al.*, 2010). Nevertheless, the HA-MRSA strains are characterized by resistance to

$\beta$ -lactam as well as other above-mentioned antibiotics. If none of the resistance mechanisms to glikopeptides is present (GISA, VRSA), antibiotics of this group are one of the last therapeutic options. Excluding glikopeptides, the HA-MRSA strains can be susceptible to rifampicin, fusidic acid and co-trimoxazole. Rifampicin and fusidic acid are rarely used as in the course of treatment strains easily acquire resistance, and co-trimoxazole is not sufficiently effective in the treatment of acute infections (DeLeo *et al.*, 2010; Gilbert *et al.*, 2011). Currently, three antibiotics recently introduced to routine medicine procedures are available and effective in treatment of HA-MRSA infections: from the oxazolidinone group — linezolid; streptogramin group — quinupristin/dalfopristin, and from the glycylicycline group — tigecycline (Appelbaum & Jacobs, 2005; Gilbert *et al.*, 2011). Thus, three therapeutic options are available in the case of multiresistant strain infections. However, they can be insufficient in specific cases i.e. the use of tigecycline in respiratory tract infections or sepsis.

## ALTERNATIVE THERAPIES

### A. Natural antibacterial compounds

New antibacterial substances are sought for and trials with known compounds of different origins are being performed. Research is carried out involving the use of natural substances with antibacterial activity obtained from bacteria (Severina *et al.*, 1998; Giacometti *et al.*, 2000; Kokai-Kun *et al.*, 2003; Nascimento *et al.*, 2006; Oh *et al.*, 2006), plants (Filipowicz *et al.*, 2003; Yin *et al.*, 2004; Yang *et al.*, 2006; Adesokan *et al.*, 2007) and animals (Giacometti *et al.*, 2000; Bexfield *et al.*, 2004; Giacometti *et al.*, 2006).

#### 1. Bacteria-derived compounds

Bacteriocins, antimicrobial peptides produced by *Staphylococcus*, which inhibit the growth of *S. aureus* are termed staphylococins. They can be produced by both *S. aureus* and *S. epidermidis*. Research on the activity of seven staphylococins against MRSA strains indicated that two bacteriocins, auricin A53 and Pep5, produced by *S. epidermidis* displayed the widest inhibition spectrum, inhibiting the growth of 87.5% of examined strains (Nascimento *et al.*, 2006). Gram-positive bacteria from the *Lactococcus* genus produce polycationic peptide bacteriocins (lantibiotics) active against both MRSA and MSSA strains (Severina *et al.*, 1998; Giacometti *et al.*, 2000; Oh

**Table 2. Toxin-mediated diseases**

| Toxin  | Toxin-mediated diseases   |
|--|---|
| TSST-1   | Menstrual Toxic Shock Syndrome (MTSS)<br>Nonmenstrual TSS (NMTSS) |
| Exfoliative toxins (ET <sub>A</sub> and B)                             | Staphylococcal Scalded Skin Syndrome (SSSS)<br>Bullous impetigo   |
| Staphylococcal enterotoxins: SEA, SEB, SEC, SED, SEE, SEG, SEH and SEI | Staphylococcal Foodborne Diseases (SFD)                           |

*et al.*, 2006). Some of them are characterized by a wide activity spectrum including Gram-positive bacteria and Gram-negative rods (Oh *et al.*, 2006). Cationic 34-residue peptides produced by *Lactococcus lactis* displayed activity against MRSA strains with a MIC<sub>90</sub> of 16 mg/L (Giacometti *et al.*, 2000). Lantantibiotics E-50-52 produced by *Enterococcus faecium* and OR-7 by *Lactobacillus salivarius* have a MIC for MDR MRSA of  $\leq 0.03$  mg/ml and 1 mg/L, respectively (Svetoch *et al.*, 2008). The commercially available lantantibiotic nisin is produced by *Lactococcus lactis* (forms A, F, Q, and Z) and *Streptococcus uberis* (forms U and U2). Field *et al.* (2008) carried out genetic modification of nisin form A and obtained derivatives N20P, M21V and K22S with elevated antibacterial activity against *S. aureus* strains, opening a new approach to staphylococcal infection therapy (Field *et al.*, 2008). In another study, nisin at 10 mg/L displayed activity towards MRSA strains and within three hours caused a 4-fold reduction of viability (Severina *et al.*, 1998). Most of the experiments were performed *in vitro* but the obtained results allow one to presume that in the future these preparations could find use in the treatment of topic infections, particularly because bacteriocins do not affect the host tissues (Kokai-Kun *et al.*, 2003; Oh *et al.*, 2006; Kokai-Kun *et al.*, 2003; De *et al.*, 2009). *In vivo* experiments conducted by De *et al.* (2009) concerned animal model infection. Rats were infected with the rate of colonisation of about  $4 \times 10^5$  viable cells and were then treated intranasally with nisin F (8192 arbitrary units). That therapy gave a protective effect in comparison with a control group and, moreover, no histopathological or morphological changes in the animals were reported (De *et al.*, 2009). Next, it has been reported that bacteriocin isolated from *Lactococcus* spp. HY 44 in the concentration of approximately 50 mg/ml (LC<sub>50</sub>) for did not show a proliferative effect on human fibroblasts and no allergic reaction in the human patch test (Oh *et al.*, 2006). Mersacidin, a 20-aminoacid lantantibiotic produced by *Bacillus* sp. HIL Y-85 54728 in a mouse rhinitis model showed antibacterial activity against MRSA. After intranasal inoculation with  $3 \times 10^2$ – $10^4$  CFU of MRSA, Mersacidin was administered intranasally twice a day over three days, giving complete eradication of MRSA. Moreover, the level of cytokines was elevated in comparison with the pre-treatment state (Kruszewska *et al.*, 2004).

Another bacteria-derived compound examined as anti-MRSA agent is lysostaphin. Originally isolated from *S. simulans* it is a glycyglycine endopeptidase capable of specifically cleaving the cross-linking pentaglycine bridges in the cell wall of *S. aureus*. Used in the form of a cream at 0.5% concentration to eradicate carriers in cotton rats, it was found more effective in comparison with 2% mupirocin or 5% nisin (Kokai-Kun *et al.*, 2003). Good therapeutic effects were obtained in experimental rabbit keratitis using 0.3% lysostaphin in eye drops and in treatment of the endophthalmitis, where 0.1% concentration was used (Dajcs *et al.*, 2002).

## 2. Plant-derived compounds

Four stilbenoids isolated from *Stemona japonica* were evaluated as antimicrobial agents. Two of them displayed high activity towards *S. aureus* (MIC 50 and 25 mg/L) comparable to that of known antibacterial compounds such as bakuchiol and magnolol (MIC 25  $\mu$ g/ml). Filipowicz *et al.* (2003) examined the activity of three components of juniper berry oil A, B and C. The highest activity against MRSA strains (MIC 1.2  $\mu$ g/ml) in comparison to a control strain ATCC 25923 was determined for fraction A comprising the highest concentra-

tion of (-)- $\alpha$ -pinene, *p*-cymene and  $\beta$ -pinene. Among three new prenylflavonoid corylifols A–C (1–3) and 13 prenylflavone derivatives isolated from *Psoralea corylifolia* seeds, nine presented activity against *S. aureus* ATCC 25923, with the MIC in the range of 0.018–0.043 mM, comparable to that of bakuchiol and magnolol, MIC 0.037 mM (Yin *et al.*, 2004). The activity of aqueous extract of *Enantia chlorantha* stem bark was examined against various microbes. The highest activity was observed against *S. aureus* (MIC 25 mg/L). Because alkaloids predominated among the solutes (46.26%), the authors presumed that they were responsible for the antibacterial activity (Adesokan *et al.*, 2007).

Flavonoids are heterocyclic compounds present in pigment plants in leaves, flowers and fruit. They are divided into 14 classes on the basis of the general structure (Cushnie & Lamb, 2011; Maia *et al.*, 2011; Oh *et al.*, 2011). They have a broad spectrum of biological activity such as antioxidant, anticancer, immunomodulatory, antiviral, antifungal and antimicrobial (Kilani-Jaziri *et al.*, 2011; Fraga *et al.*, 2010; Ozcelik *et al.*, 2011). Bactericidal activity of flavonoids has been reported against several bacterial species, including *S. aureus* (Verdrengh *et al.*, 2004; Hong *et al.*, 2006; Kilani-Jaziri *et al.*, 2011; Ozcelik *et al.*, 2011), but the mechanism of that activity is still poorly understood (Cushnie & Lamb, 2011). Kilani-Jaziri *et al.* (2011) studied Total Oligomer Flavonoids (TOF) extract obtained from *Cyperus rotundus* and found its MIC for *S. aureus* was 0.5 mg/ml and MBC 1 mg/ml. As a control, ampicillin was used (MIC 0.0015 mg/ml and MBC 0.225 mg/ml) (Kilani-Jaziri *et al.*, 2011). In our opinion, it is extremely difficult to evaluate the real bactericidal activity of a novel substance on the basis of such investigations on a highly sensitive and non- $\beta$ -lactamase producing strains as is indicated by its low ampicillin MIC. Hong *et al.* (2006) studied the inhibitory activity of genistein, a radioprotective soy isoflavone, against opportunistic *S. aureus* that could cause sepsis in patients after radiotherapy. *S. aureus* culture ( $1 \times 10^3$  CFU/ml) was treated with 100  $\mu$ M genistein and after 8 h of incubation a significant reduction in viable counts was reported ( $p < 0.05$ ). Moreover, genistein had no bactericidal activity against probiotic *Lactobacillus reuteri* species (Hong *et al.*, 2006). These promising results suggest that genistein with probiotic bacteria could enhance antibiotic therapy in the case of infections in such high risk patients. Verdrengh *et al.* (2004) revealed that the bactericidal activity of genistein against *S. aureus* is due to its inhibitory action towards topoisomerase IV affecting DNA metabolism. Wang *et al.* (2010) proved that the antibacterial activity of soybean isoflavone (SI) against *S. aureus* strains results from inhibition of topoisomerases I and II. The activity of the topoisomerases was inhibited by SI at 6.4 mg/ml. The antimicrobial activity of flavonoids could be due to direct inhibition of bacterial growth as well as to the inhibition of virulence mechanisms (Oh *et al.*, 2011; Budzyska *et al.*, 2011). Oh *et al.* (2011) investigated 14 flavonoids extracted from *Sophora flavescens* roots and their ability to inhibit bacterial growth and the activity of bacterial sortase A. Sortase A (SrtA) is a key enzyme involved in the pathogenicity of *S. aureus*. The chemical pHMBC known for its inhibitory property toward SrtA (IC<sub>50</sub> = 130  $\mu$ M) served as a positive control. The highest activity was assigned to kurarinol (IC<sub>50</sub> =  $107 \pm 6.6$   $\mu$ M), but despite that all of the flavonoids studied revealed weak activity against *S. aureus* growth.

A serious problem especially of chronic infections is formation by microorganisms of biofilm, which is difficult to eradicate with standard antibiotic therapy.

Thus, researchers are still looking for an alternative options to eliminate the biofilm-forming microorganisms. Budzyńska *et al.* (2011) studied 22 synthetic flavonoids of which three 3-arylidene flavones, 2b, 2c, and 2i, revealed efficient antimicrobial activity against *S. aureus*. Interestingly, 3-arylidene flavone 2c inhibited the initial adhesion of bacteria to abiotic surfaces which resulted in blocking the biofilm formation. Moreover, those authors proved that due to the lipophilic nature the 3-arylidene flavones influence the cell-membrane integrity causing the bacterial growth inhibition. Another mechanism of antimicrobial activity of flavonoids is modulation of antibiotic resistance (Cushnie & Lamb, 2011; Maia *et al.*, 2011). Maia *et al.* (2011) revealed that six flavones isolated from *Praaxelis clematidea*. King and Robinson inhibited staphylococcal NorA efflux pump decreasing the norfloxacin MIC 16-fold in a reference strain of *S. aureus* SA-1199B. However, inhibition of bacterial growth was not reported. Some flavonoids reduce the oxacillin MIC towards MRSA strains, thereby enhancing their susceptibility to oxacillin and other  $\beta$ -lactam antibiotics. Epicatechin gallate reduces the MIC value 256–512-fold and the flavone baicalein up to 1024-fold (Cushnie & Lamb, 2011).

### 3. Animal-derived compounds

Renalexin, a 20-residue peptide isolated from skin of the bullfrog *Rana catesbeiana* displayed activity against clinical MRSA strains (MIC<sub>90</sub> 16  $\mu$ g/ml) (Giacometti *et al.*, 2000). A native thermostable antibacterial factor isolated from third-instar larvae of *Lucilia sericata*, composed of two fractions <500 Da and 0.5–3 kDa, demonstrated activity against MRSA and MSSA strains as well as Gram-negative rods (Bexfield *et al.*, 2004). Distinctin, a protein isolated from *Pollymedusa distincta*, displayed a synergistic effect with glycopeptides towards biofilms in *in vitro* experiments and in an animal model of *S. aureus*-infected central venous catheter (CVC). The bacterial load in the biofilm decreased to 10 CFU/ml (Giacometti *et al.*, 2006).

The natural amphiphilic steroids squalamine and MSI-1436 from the dogfish shark *Squalus acanthias* exhibited the best antimicrobial activity against *S. aureus* ATCC 29231 (1–2  $\mu$ g/ml) comparing to other microorganisms, as *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Candida albicans* (MIC = 4–8  $\mu$ g/ml) (Brunel *et al.*, 2005). Since these natural amino sterols are difficult and expensive to obtain, new derivatives have been synthesized showing antimicrobial activity against *S. aureus* with a MIC from 1.56 to 6.25  $\mu$ g/ml comparing to the MIC for squalamine <3.125  $\mu$ g/ml (Salmi *et al.*, 2008a; 2008b). Against MRSA the MIC of those compound ranged between 1.25 and 5  $\mu$ g/ml as compared to 2.5  $\mu$ g/ml for squalamine (Salmi *et al.*, 2008b).

Chitosan (poly-D-glucosamine) is a natural polymer obtained from chitin, which occurs in exoskeletons of shellfish and insects. Chemical modification by acetylation results in increased water solubility (Chi *et al.*, 2006). Due to its polycationic character, chitosan derivatives display bactericidal activity against different microorganisms including *S. aureus* strains. At 0.63 g/L, a chitosan derivative inactivated 99.8% of studied *S. aureus* strains *in vitro* (Chi *et al.*, 2007). According to Runarsson *et al.* (2007), the MICs for methylated derivatives of chitosan towards *S. aureus* strains were lower at pH 5.5 than at pH 7.2 and ranged from 16 to 512  $\mu$ g/ml. *In vivo* experiments using a mouse model of *S. aureus*-infected wounds confirmed the effectiveness of chitosan acetate bandage improving wound healing (Burkatovskaya *et al.*, 2008).

Propolis is a waxy resinous hive product collected by *Apis mellifera* bees from tree buds and green plants mixed with secreted bee enzymes. It is used to seal up holes and cracks in the hive. The chemical content of propolis depends on geographical region and plants that grow in the apiary surrounding. The main components responsible for its biological activity (antimicrobial, antifungal, antiviral, anti-tumor and immunomodulatory) are polyphenols and flavonoids (Sforzin & Bankova, 2011; Chaillou & Nazareno, 2009). Promising results for MRSA eradication were displayed by ethanolic extract of propolis acting synergistically with mupirocin. In a rabbit model animals treated with combination of mupirocin and propolis displayed significantly lower bacterial cell counts than animals receiving either treatment alone ( $p=0.0001$ ). Moreover, the significant reduction of polymorphonuclear leukocytes count on nasal mucosal membranes was reported ( $p<0.05$ ) (Onlen *et al.*, 2007).

The majority of natural compounds and their derivatives display properties that make them promising candidates for topical rather than systemic drugs. The lack of *in vivo* toxicity bodes well for their usefulness in clinical practice. Nonetheless, further studies are required.

### B. Old medications — new uses

Numerous medicinal compounds used for other purposes are currently being studied for possible antibacterial activity. The aim of these studies is to evaluate their bactericidal activity as well as the possible synergistic action with antibiotics and other antibacterial chemotherapeutics through inhibition of bacterial drug resistance mechanisms. Polyoxotungstates (POT), which sensitizes MRSA strains to beta-lactam antibiotics through the reduction of PBP2a expression, exemplifies such mechanisms. The mechanism responsible for this phenomenon is probably the stress response of MRSA to POT. Sensitization to beta-lactams was also seen with other substances such as fosfomycin, Triton X-100, tannin and some flavonoids (Tajima, 2005).

The antipsychotic drug thioridazine inhibits efflux pump efficiency. The addition of thioridazine to norfloxacin for MRSA lead from 2 to 8-fold reduction of MIC for norfloxacin and oxacillin (Kristiansen *et al.*, 2006). Thioridazine itself displays bactericidal activity against *S. aureus*. However, the concentration of thioridazine in blood serum that is required to achieve such bactericidal effect is higher than can be achieved clinically. Thus this compound is more likely to be used synergistically with antibiotics to treat infections with strains with active efflux pumps (Thanacoody, 2007).

Sandrini *et al.* (2007) examined the antibacterial activity of two nucleoside analogue drugs, antiviral AZT (azidothymine) and anticancer Gemcitabine (2',2'-difluoro-2'-deoxycytidine, dFdC). These analogues are specifically activated by endogenous bacterial deoxyribonucleoside kinases, which results in death of the bacteria. Among the examined AZT analogues, 5-fluoro-2'-deoxyuridine (FdUrd) displayed the highest antibacterial activity, with the MIC for *S. aureus* ATCC 29213 and CCM 885 of 0.00316  $\mu$ M and 0.01  $\mu$ M, respectively. The MIC for gemcitabine was 0.01  $\mu$ M and 1.0  $\mu$ M, respectively.

### C. Cationic Antimicrobial Peptides

Cationic antimicrobial peptides (CAMPs) such as defensins, cathelicidin and thrombocidins are positively charged (+2 to +7) amphipathic molecules with antibacterial properties (Peschel & Collins, 2001; Zanetti *et al.*, 2002; Hiemstra *et al.*, 2004). Produced by the innate

immune system they protect the skin and epithelia from the bacteria invasion (Stryjewski *et al.*, 2007). They play a key role in the innate immune response and help in the functioning of neutrophils and platelets, showing a dual mode of action — directly owing to their antibacterial activity and indirectly, by activation of components of the immune system (Zanetti *et al.*, 2002; Pei, 2004). Their mechanism of action is based on their amphipathic hydrophobic and hydrophilic nature which is important for the initial interactions with the bacterial membrane that lead to the disruption of ion homeostasis (Peschel & Collins, 2001; Peschel, 2002; Pei, 2004). Research is carried out regarding the use in therapy of natural peptides and their synthetic analogues (Benincasa *et al.*, 2003; Giacometti *et al.*, 2004; Cirioni *et al.*, 2006a; Ghiselli *et al.*, 2007) and other compounds with similar characteristics and modes of action (Jin *et al.*, 2005; Chin *et al.*, 2007).

Protegrins were originally isolated from porcine leukocytes. They are 16–18-residue cysteine-rich peptides (Zanetti *et al.*, 2002). IB-367 is a synthetic protegrin used in co-treatment of *S. aureus* infections with linezolid in an experimental model of CVC-associated infections (Ghiselli *et al.*, 2007). When a catheter was treated with IB-367 at 10 µg/ml with linezolid (32 µg/ml) a decrease in the number of cells in blood culture from  $7.8 \times 10^4$  CFU/ml to  $10^1$  CFU/ml was observed and in the catheters/venous tissue culture the decrease was from  $7.5 \times 10^5$  CFU/g to  $4.8 \times 10^2$  CFU/g. In *in vitro* experiments the MIC of linezolid for *S. aureus* in the presence of IB-367 decreased four times in comparison with a culture without IB-367 and it was at the same level as for planktonic cells (Ghiselli *et al.*, 2007). Similar research was conducted by Cirioni *et al.*, (2006a) who pre-treated central venous catheters with the 27-residue cathelicidin BMAP-28. In *in vivo* studies, when CVC were treated with BMAP-28 and subsequently with vancomycin or linezolid a reduction in the biofilm bacterial load from  $10^3$  to  $10^1$  CFU/ml was obtained and bacteremia became undetectable. In the presence of BMAP-28, the *in vitro* MIC for linezolid decreased 4-fold. In experimental acute peritonitis in mice caused by MRSA, BMAP-28 treatment reduced the mortality to control values (Benincasa *et al.*, 2003). BMAP-28 not only acted synergistically with antibiotics reducing their MIC but also protected mice from the development of sepsis. When live *S. aureus* cells were administered to mice and a combined antibiotic and BMAP-28 therapy was applied, the factors induced a similar reduction of mortality in infected mice. When dead cells were administered only, BMAP reduced mortality due to decreasing the level of tumor necrosis factor- $\alpha$  and interleukin-6 in the serum, thus indicating its ability to inhibit the development of septic shock (Giacometti *et al.*, 2004).

Caregnins CSA-8 and CSA-13 are synthetic cationic steroid molecules which resemble in their mode of action endogenous antimicrobial peptides. In tests towards 50 clinical glycopeptide heterointermediate *S. aureus* (hGISA) and glycopeptide-intermediate *S. aureus* (GISA) strains and four VRSA ones CSA-13 showed high activity with a 4-fold lower MIC (1 µg/ml) for VRSA in comparison with CSA-8. The MIC<sub>90</sub> for hGISA and GISA was 1 µg/ml and for CSA-8, 8 µg/ml (Chin *et al.*, 2007). Urokinase-type plasminogen activator (uPA) is a serine protease produced in an inactive form mainly by the kidneys. The activated form has fibrinolytic function and modulates the innate immune response. In *in vitro* and *in vivo* experiments, uPA displayed antibacterial activity towards *S. aureus*, therefore it acted as an endogenous

antibiotic and the effect was dose-dependent (Jin *et al.*, 2005).

Unlike the previously described compounds, endogenous peptides seem to have potential in parenteral therapy. However, similarly to most compounds with antibacterial activity there is a risk of a lack of efficacy towards strains inducing infections. The negative net charge of both staphylococcal teichoic acid and phospholipids plays a key role in the susceptibility to CAMPs (Peschel & Collins, 2001; Peschel, 2002; Hiemstra *et al.*, 2004). Due to the fact that most endogenous peptides have a positive charge, ionic forces lead to the accumulation of the peptides in the cell wall, interactions with the surface of the cell membrane, integration with the lipid layer and pore formation (Peschel & Collins, 2001; Peschel, 2002; Hiemstra *et al.*, 2004). It is recognised that acquiring resistance to cationic peptides is based on the modulation of the electrical charge of the wall and cell membrane, which leads to the reduction in the interactions with the peptide antibiotics. This can take place by modification of the teichoic acid in the cell wall by incorporation of D-alanine or modification of phosphatidylglycerol by L-lysine as well as by efflux pump (*gacA*) activation (Peschel, 2002).

Dendrimers, low molecular mass peptides are synthetic analogs of natural, linear antimicrobial peptides, but have a branched structure. Their mechanism of action is similar to that of cationic peptides and is based on interactions with the bacterial membrane. Janiszewska and Urbanczyk-Lipkowska (2006) studied various dendrimers and found that the MICs for *S. aureus* ranged from 64 µM for dendrimer 169, –69 µM for dendrimer P13, –75 µM for dendrimer 155 and to –144 µM for P2. When tested against *S. aureus*, *E. coli* and *C. albicans*, dendrimer P13 was the most effective overall with a MIC of 69, 32 and 69 µM, respectively. However, against *S. aureus* specifically, dendrimer 169 was the most effective.

Current knowledge indicates that peptide antibiotics should be viewed as highly potent agents for treatment of multidrug resistant microorganisms-related infections. Moreover, it is suggested that peptide antibiotics, unlike the bacteria-originated antimicrobial agents, could display a lower potential for suppression of the physiological flora. Synthetic analogs of endogenously produced peptides can be chemically modified to modulate their effectiveness and eliminate produced side-effects. Moreover, the antimicrobial activity as well as ability to modulate host immune response make the peptide antibiotics highly promising. Such chemical modifications lead to inhibition of septic shock syndrome due to reducing the level of septic shock mediators. Such effect could not be obtained with the use of other groups of antibiotics. Peptides could activate the host cellular immune response, which is extremely important in the case of multifactorial pathogenesis of staphylococcal infections. Obviously, acquisition of resistance to peptide antibiotics can also take place, thus susceptibility testing seems to be required before the treatment.

## D. Prevention of biofilm formation

A protection strategy against biofilm formation is based on the prevention of staphylococci adhesion to the host cell or to the surface of synthetic materials used in various types of catheters and implants (Balaban *et al.*, 2003; Dell'Acqua *et al.*, 2004; Cirioni *et al.*, 2006a; Balaban *et al.*, 2007). Introducing new less hydrophobic biomaterials (Campoccia *et al.*, 2006) as well as covering their surface with antibiotics and antiseptics can pre-

vent the adhesion of biofilm-forming bacteria (Richards *et al.*, 2006; Ghiselli *et al.*, 2007; Bahna *et al.*, 2007). As far as biofilm production is concerned, disruption of quorum sensing as well as the expression of virulence factors with RNAIII-Inhibiting Peptide (RIP) reduced the growth of bacteria and allowed access of antibiotics (Balaban *et al.*, 2001; 2003; Korem *et al.*, 2003; Cirioni *et al.*, 2006b). RIP is a hexapeptide, which in *S. aureus* inhibits the synthesis of toxins by interference with the RNAIII Activating Protein (RAP) (Korem *et al.*, 2003). The RAP is a protein which activates the production of many toxins through phosphorylation of the RAP target — TRAP. If its expression or phosphorylation is disrupted by the RIP, the expression of the virulence regulator, *agr* gene, is inhibited and „quorum sensing” is blocked (Korem *et al.*, 2003; Cirioni *et al.*, 2006b). Balaban *et al.*, (2003) reported that the RIP inhibited the adhesion to HaCat (human skin keratinocytes) and Hep-2 (human epithelial cells) and also reduced the adhesion to dialysis catheters, both those made of polyurethane and of silicone. Moreover, RIP was found more effective against strains with a high adhesion affinity (Balaban *et al.*, 2003). *In vitro* studies revealed that the treatment of *S. aureus* cells in a biofilm with RIP and subsequent addition of an antibiotic produced a significant decrease in the MICs and MBCs to values comparable to those obtained for planktonic cells. It was shown that the RIP alone displayed no bacteriostatic or bactericidal activity. These results were confirmed in an *in vivo* model of rats with staphylococci CVC (central venous catheter) infection. The application of RIP and antibiotics greatly reduced the bacterial load in the biofilm by 6 log<sub>10</sub> (Cirioni *et al.*, 2006b). In research carried out by Balaban *et al.*, (2000), bacteria pre-incubated with the RIP were used to induce infection *in vivo*. Strains treated with the RIP caused less frequent infections than untreated strains — by 40% and 58% for keratitis and osteomyelitis in rabbits, respectively; by 60% for septic arthritis in mice and by 70–100% for mastitis in cows.

Simonetti *et al.*, (2008) revealed that in the case of MRSA-infected wounds dressing soaked with RIP significantly enhanced wound healing in mice. Additionally, also mice treated intraperitoneally with teicoplanin showed better healing. No side effects were observed.

The therapeutic strategy based on counteracting the biofilm formation seems to be more promising and safe than biofilm eradication in the course of treatment. The simultaneous administration of antibiotics and RIP inhibiting quorum sensing revealed a synergistic action in biofilm reduction. Moreover, *in vivo* studies indicate that such an approach could be used in the treatment of local as well as generalized infections treatment.

## E. Bacteriophages

Until now, bacteriophages have been used in therapy mainly in Eastern European countries and the former Soviet Union. There are no formal regulations and standards for biotheriophage therapy despite their use in a large number of patients (Keilman, 1979; Slopek *et al.*, 1987; Sulakvelidze *et al.*, 2001; Parfitt, 2005). As described by Verbeken *et al.* (2007), the studied therapies must be in accordance with paragraph 32 of the Declaration of Helsinki and under supervision of an Ethics Committee. In June 2007, a limited clinical trial was approved by an Ethics Committee to be performed in the Burn Wound Center in Belgium (Verbeken *et al.*, 2007). In 2005 in Poland, the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences

began experimental treatment of outpatients. Three formal conditions had to be met, namely: approval by an institutional review board, a written informed consent of patients, and insurance (Gorski *et al.*, 2009). There is still a lack of well documented research including controls, therefore robust conclusions as to the usefulness and dangers of bacteriophage therapy cannot be made (Sulakvelidze *et al.*, 2001). Nevertheless, phage therapy seems to be worth further investigation due to its underlying concept. Phage selectively kills only the target bacteria and in the case of *S. aureus* infections both MSSA and MRSA strains are susceptible (Matsuzaki *et al.*, 2003; Capparelli *et al.*, 2007). While the antimicrobial activity of an antibiotic includes both pathogenic bacteria as well as the physiological flora, a key problem of phage therapy is selection of a phage specific to different strains of a certain species. Sulakvelidze *et al.*, (2001) mention other advantages of phage therapy. Phages replicate in the infection site and do not cause side-effects, moreover, bacteria that are resistant to some phages can be sensitive towards others.

A drawback of this sort of therapy can be a narrow spectrum of sensitive strains, which can require the use of a mixture of phages. It is also essential to select lysogenic phages due to the risk of transferring genes encoding toxins and resistance to antibiotics (Sulakvelidze *et al.*, 2001). Parfitt (2005) describes the clinical use of phage therapy in Georgia, where this therapy helped to reach a clinical state enabling plastic surgery in three patients with purulent staphylococci-infected burns caused by a radioactive agent. This therapy is based on using a synthetic skin soaked in phages, from which they are slowly released to the environment of the wound as a consequence of slow biodegradation of the artificial skin (Parfitt, 2005). Keilman (1979) presented results regarding the local treatment of purulent bone inflammation. Polyvalent phages were used in combination with locally applied antiseptics parallel to surgical intervention and antibiotic treatment. As a result a reduction in purulent complications from 23.2% to 4.8% was observed. New research regarding this subject has also been presented in several publications (Matsuzaki *et al.*, 2003; Wills *et al.*, 2005; Capparelli *et al.*, 2007). In an experimental treatment of abscesses in rabbits with the LS2a phages, a significant eradication of infection was obtained (Wills *et al.*, 2005) and the use of the MR11 phage to treat MRSA peritonitis in mice prevented death up to seven days (after this time they were killed), whereas in the non-treated control group the mortality after 24 h was above 90% (Matsuzaki *et al.*, 2003). The MR11 phage was selected due to its widest spectrum towards various *S. aureus* strains and also because it does not transfer toxin and resistance genes (Matsuzaki *et al.*, 2003). A suspension of M<sup>Sau</sup> phages brought about total infection eradication in mice and only 3% (1/30) died. This phage also has a wide spectrum of activity towards many *S. aureus* strains including MRSA. In a control (non-treated) group 100% (30/30) mice died ( $p < 0.0001$ ) (Capparelli *et al.*, 2007). In a model of catheter-related infection, λ80 phage reduced the optical density of a 24 h biofilm by 79.4% (Del Pozo *et al.*, 2007). Leszczyński *et al.*, (2006) described the use of phages in eradication of MRSA carriage in a health worker. A nurse, who suffered a urinary tract infection caused by MRSA, after a successful treatment became carrier in the digestive tract. She refused eradication with antibiotics but agreed to bacteriophage treatment. Phage lysate was administered orally and decolonization was completed successfully.

Phages have also been used as vectors to increase the efficacy of therapy (Embleton *et al.*, 2005; Yacoby *et al.*, 2007). Yacoby *et al.*, (2007) reported the use of phages in the transfer of antibiotic molecules to the bacterial cell. During the first stage of the research, trials involved the direct transport of chloramphenicol with the help of filamentous bacteriophages to *S. aureus* cells. However, the effect of bacterial growth inhibition was only partial due to the transfer of a small amount of the drug caused by its hydrophobicity. Bacteriophage 75 complex was used to transfer a photosensitizer and light-activated antimicrobial agents to *S. aureus* cells. On exposure to light, this complex killed different MSSA, MRSA and VISA cells. The use of a control consisting of phage only showed that the killing effect was not a consequence of bacteria lysis by the phage (Embleton *et al.*, 2005).

The current knowledge suggests that bacteriophage-related therapy should mainly concern the treatment of local infections as well as surveillance eradication especially in the case of drug-resistant pathogens. Nevertheless, *in vivo* studies indicate that in the nearest future phage-related therapy will not be applied to severe multiorgan infections or generalized infections. Such cases would prompt the host immune system to eliminate bacteriophages as a foreign agents before their could act against the pathogens. Moreover, it is hardly possible for a bacteriophage culture to be standardized as viruses mutate easily. Next, this alternative approach involves three live components — patient, bacteria and virus — thus the final result could be difficult to predict. Undoubtedly, the obvious benefits of phage-related therapy in comparison with antibiotic therapy include low cost and relatively short time of preparation of new agents.

#### F. Photodynamic therapy

Photodynamic therapy (PDT) is based on the use of chemicals termed photosensitizers. Photosensitizers accumulate in specifically selected tissues or cells and their activation is a result of the activity light of an adequate wavelength. This leads to the generation of singlet oxygen and free radicals responsible for the cytotoxic effect towards specific cells (Hamblin *et al.*, 2002; Romanova *et al.*, 2003; Mohr & Redecker-Klein, 2003; Jori and Brown 2004). Some mechanisms responsible for the cytotoxic effect have been examined and characterized. Depending on the photosensitizer used, the mechanism can be associated with cell wall disruption, inactivation of enzymes or indirect damage to the genetic material of the bacteria (genomic and plasmid DNA) (Romanova *et al.*, 2003; Sharma *et al.*, 2008).

Up to now mainly preliminary investigations have been performed, such as analyzing the survival rate of bacteria *in vitro*, depending on the type and dose of the photosensitizer, wavelength and illumination duration (Maisch *et al.*, 2005; Grinholc *et al.*, 2007a; 2008a; 2008b; Sharma *et al.*, 2008; Peloi *et al.*, 2008).

From a clinical point of view, an important issue is easy killing of the multi-resistant MRSA strains in response to photodynamic therapy as development of resistance to this therapy seems to be unlikely, in contrast to the quick gaining of resistance towards different antibiotics and other antibacterial agents (Hamblin & Hasan, 2004; Grinholc *et al.*, 2007a).

Grinholc *et al.* (2008b) reported that the response to photodynamic inactivation with protoporphyrin diarginate (PPArg<sub>2</sub>) was different among various clinical MSSA and MRSA strains and ranged from 0 to 3 log<sub>10</sub> unit reduction in viable counts. Assuming a reduction of 1–2

log<sub>10</sub> units as criteria of sensitivity and reduction of 2–3 log<sub>10</sub> units as high sensitivity, 62.5% of MSSA and 40% of MRSA were sensitive and multidrug-resistance correlated with resistance to photodynamic therapy ( $p < 0.05$ ). Comparing *in vitro* biofilm-forming strains with non-biofilm-forming strains, a higher killing efficacy was observed in the latter. Among the highly sensitive strains ( $> 2$  log<sub>10</sub>) 75% did not produce a biofilm (Grinholc *et al.*, 2008b). It is worth to emphasize that susceptibility of different strains to photodynamic inactivation was not studied earlier and investigations along these lines should be continued. Determination of molecular markers predicting strain response to photo-inactivation would lead to more effective treatment (Grinholc *et al.*, 2008b). Sharma *et al.* (2008) reported high *in vitro* efficacy of toluidine blue (TBO) used with laser light in the reduction of MRSA biofilm, where the reduction effect was dependent upon the light dose (Sharma *et al.*, 2008). Recent discoveries showed that sensitivity of *S. aureus* strains to broadband visible light is also strain-dependent indicating that the level of hydroxyl and superoxide radical production determines the strains' response to photo-inactivation (Lipovsky *et al.*, 2009).

So far, few papers on *in vivo* studies have been published (Soukos *et al.*, 1998; Komerik *et al.*, 2003; Wilson 2004). On the basis of the results obtained by Bisland *et al.* (2006) on animal models, it can be assumed that photodynamic therapy is potentially one of the best therapeutic options for treating osteomyelitis of *S. aureus* etiology, especially that these infections are associated with a biofilm production. An obvious advantage of photodynamic therapy stems from its mechanism involving singlet oxygen generation that makes it impossible to induce effective resistance mechanisms (Wilson, 2004). Importantly, unlike antibiotic treatment photodynamic therapy does not influence the physiological bacterial flora (Romanov *et al.*, 2001; Wilson, 2004) owing the possibility of specific introduction of the photosensitizer to selected bacterial cells and also to the restricted illumination area (Hamblin & Hasan, 2004). An additional advantage of this method is its low cytotoxic effect towards host cells along with the high efficacy towards pathogens (Soncin *et al.*, 2002; Grinholc *et al.*, 2008a) and also a lack of any direct or delayed damage to the genetic material (Zeina *et al.*, 2002; 2003).

#### G. Staphylococcal vaccines

Literature regarding staphylococcal vaccines is very comprehensive, however, there is still a problem with the introduction of a efficient vaccine in the prevention of infections caused by *S. aureus*. This can be associated with the fact that a human organism throughout its life is in contact with this bacterium as many people are constant or transient carriers of *S. aureus* on the mucus of the nasal or oral cavity. The selection of patients for this preventative therapy should be based on recognizing the risk factors and should be preceded by research to allow individualized approach (Fattom *et al.*, 2004; Schaffer & Lee, 2008). Earlier the whole bacterial cell was used as an antigen, but recent research is aiming at obtaining antibodies against specific virulence factors such as adhesins, toxins or invasins (Otto, 2008). Despite the multicomponent regulation of pathogenesis, interference with any component can reduce virulence and symptoms of disease (Balaban *et al.*, 2000).

Nonetheless, despite the substantial progress in recent years, no vaccine has yet been approved and in fact only one, StaphVAX<sup>®</sup>, successfully underwent clinical trials

(Deresinski, 2006; Schaffer & Lee, 2008). StaphVAX<sup>R</sup> is a bivalent vaccine composed of *S. aureus* capsular polysaccharide (CPS) types 5 and 8 conjugated with a non-toxic *Pseudomonas aeruginosa* exotoxin (Fattom *et al.*, 2004). These antigens were chosen because these capsular types are the most often described for strains isolated from infected patients (Otto, 2008; Sadowska *et al.*, 2001; Deresinski, 2006). However, clinical phase II and III studies indicate that the vaccine offers only a partial protection from bacteremia lasting for a few weeks in 60% of immunized patients (Shinefield, 2006). Due to the planned expansion of the activity spectrum of the vaccine to *S. aureus* 336, *S. epidermidis* PS-1, *S. epidermidis* GP-1 and PVL antigens, Nabi Biopharmaceuticals suspended the research (Deresinski, 2006). Currently, Merck conducts clinical phase II trials concerning vaccine V710 (previously designated 0657nl), where the antigen used is a cell-wall-anchored IsdB protein (Schaffer & Lee, 2008). Iron surface determinant B (IsdB) is an iron-sequestering protein conserved in various clinical MSSA and MRSA strains. It is characterised by an iron binding ability that enables bacteria to obtain it from the environment. Its expression occurs when iron level is low in the growth environment of the bacteria (Kuklin *et al.*, 2006). Clinical trials are conducted with patients undergoing hemodialysis and cardiosurgery (Schaffer & Lee, 2008).

The IsdB protein perfectly exemplify the direction of effort to create a vaccine against *S. aureus* vaccine. IsdB is present in all clinical *S. aureus* strains, thus the antimicrobial effectiveness of an IsdB-based vaccine is suggested to be much higher than in the case of vaccines targeting capsular polysaccharide (CPS) types 5 and 8. Moreover, IsdB as a protein should trigger significantly higher immunological response than capsular polysaccharides. Such vaccines would not be effective against bacteria bearing other serotypes of CPS.

Clinical phase II trials are also underway for some drugs used for passive immunization comprising immunoglobulins against ClfA (Tefbazumab [Arexis] — company Inhibitex), ABC transporter GrfA (Aurograb — NeuTec), lipoteichoic acid (Pagibamaximab [BSYX-A110] — Biosynexus) and CP5 and CP8 (AlfaStaph — Nabi). The lack of a clinically approved vaccine against *S. aureus* justifies the ongoing search for novel antigenic determinants in the bacterial cell as potential vaccine targets.

The MSCRAMM adhesion proteins are located on the surface of the cell. Antibodies directed against them could block the binding of the microorganism to a host tissue preventing colonization and infection initiation (Otto, 2008). Research is carried out in two directions, namely immunization by application of the surface proteins and in consequence induction of an immune response (Rennermalm *et al.*, 2001; Josefsson *et al.*, 2001; Shannon *et al.*, 2006; Kuklin *et al.*, 2006; Zhou *et al.*, 2006) as well as by DNA vaccines comprising genes encoding specific surface proteins. DNA vaccines induce the expression of encoded proteins in eukaryotic cells, thereby generating an immune response in the form of specific antibodies. As shown, these proteins can also modify the cell-mediated immune response (Brouillette *et al.*, 2002; Kerro-Dego *et al.*, 2006; Gaudreau *et al.*, 2007; Therrien *et al.*, 2007).

In many *in vivo* studies interesting results have been obtained. When Extracellular Fibrynogen-Binding Protein (Efb) was used for immunization of mice with foreign-body-associated wound infection, it induced a strong immune response that reduced the severity of the infection. Only 17% of mice developed a serious infec-

tion in comparison to 73% in the control group (Shannon *et al.*, 2006). Josefsson *et al.* (2001) reported that in experimental septic arthritis in mice, immunization with recombinant ClfA induced a less severe course of disease, and passive immunization with rat or rabbit anti-ClfA antibodies protected against severe arthritis and sepsis-induced death. ClfB, a protein binding cytokeratin-10, plays an important role in the colonization of the nasal passage, a known risk factor for *S. aureus* infection. Mice immunized systemically or intranasally with a vaccine containing the A domain of ClfB displayed a lower level of colonization than a control group. A vaccine containing a truncated D2-domain of fibronectin-binding protein (FnBP) displayed on a cow-pea mosaic virus carrier induced protection against endocarditis in a rat model (Rennermalm *et al.*, 2001). Application with an adjuvant induced a strong response in mice protecting them from infection and sepsis and in rhesus macaques a five-fold increase in the production of antibodies was observed after a single dose (Kuklin *et al.*, 2006). Both single surface-proteins as well as conjugated proteins can serve as antigens. Due to the potentially wider protection spectrum and stronger immune response research on conjugated vaccines containing two or more antigens is being carried out. A recombinant vaccine containing Cna-FnBP induced a strong humoral response which was the highest around 5 and 6 weeks after immunization. This resulted in a higher survival rate of infected mice in comparison to a group immunized with Cna or FnB individually (Zhou *et al.*, 2006).

#### 1. Polyvalent vaccines

In a mouse model of abscess formation, immunization with a vaccine containing four proteins IsdA, IsdB, SdrD, and SdrE induced complete protection in contrast to only partial one obtained with any single antigen. These four antigens were selected as they induced the highest level of antibodies among fifteen tested. A positive correlation was observed between the level of antibodies and opsonophagocytic properties (Stranger-Jones *et al.*, 2006). Conjugated vaccines were also examined in the context of DNA-mediated immunization and expression of the products of genes. The immune response in mice was evaluated after DNA vaccination with plasmids encoding an individual antigen or a chimeric protein composed of the surface-located GapB and GapC proteins. The recombinant vaccine induced a stronger humoral response than a vaccine bearing either single gene (Kerro-Dego *et al.*, 2006). For a polyvalent vaccine containing genes encoding the sortase enzyme (ClfA-FnBPA-Srt), the combination of genes induced a better response than any of the genes alone. Moreover, the conjugated vaccine induced a T1 and T2 response giving a protective effect from the complex pathogenesis of *S. aureus*. After 21 days post-infection, 55% of mice immunized with the multi-gene vaccine survived in comparison to a control group where only 15% survived (Gaudreau *et al.*, 2007). However, not all of the obtained results regarding vaccines containing surface proteins are clear. Therrien *et al.* (2007) reported that immunization with a DNA vaccine containing the *cna* gene did not protect mice from a lethal-induced infection. In fact, the only mice that died in the experiment were the immunized ones.

#### 2. Vaccines against biofilm

A surface polysaccharide poly-*N*-acetylglucosamine (PNAG), present in *S. aureus* and *S. epidermidis*, partici-

pates in biofilm production (Maira-Litran *et al.*, 2004; Perez *et al.*, 2009). Its production is encoded by a gene from the *ica* locus, which is present in most clinical *S. aureus* isolates. This compound displays immunogenic properties and therefore could be a good vaccine candidate (McKenney *et al.*, 1999; Maira-Litran *et al.*, 2004; Maira-Litran *et al.*, 2005). In a mouse renal infection model the application of PNAG induced a high level of IgG antibodies (>500 UI/ml). Rabbits immunized with PNAG produced even higher levels of antibodies (>2500 UI/ml) at a constant level for at least 8 months. Comparing the efficacy of PNAG and its deacylated (dPNAG) form conjugated with diphtheria toxoid (DT) carrier protein used to immunize animals, a marked increase in the level of antibodies was obtained with dPNAG as opposed to native PNAG (Maira-Litran *et al.*, 2005). Because the efficacy of antibodies against a carbohydrate vaccine may not include all *S. aureus* strains as not all express this polysaccharide despite the presence of the *ica* locus (Grinholc *et al.*, 2007b), new candidates for the production of an efficient vaccine in chronic infection therapy and prevention are being sought for. Proteins immunogenic in MRSA-induced chronic osteomyelitis with biofilm formation were examined in a rabbit model of tibial infection. Among the 22 antigens identified, antibodies for some of them such as lipase, autolysin or lipoprotein were produced at an early stage on the 14<sup>th</sup> day of infection. In the case of other antigens, transketolase or elongation factor, antibodies were produced during the late stages of infection on the 42<sup>nd</sup> day. The authors suggest that antigens that are recognized earlier are good vaccine candidates and those recognized in late stages can be used in adjuvant therapy (Brady *et al.*, 2006).

PNAG is a weak immunogen, so increasing the effectiveness of vaccination, especially with the use of antigens secreted at different stages of biofilm formation taking advantage of the above considerations is an attractive option. Investigations aimed at identification of patients that should be vaccinated and those that should be administered with an adjuvant are a prerequisite. They should be based on detailed bacteriological and serological diagnoses.

### 3. Vaccines against extracellular proteins

Another approach involves the use of vaccines against extracellularly secreted proteins with superantigen (exotoxin) properties such as TSST-1, enterotoxin B (LeClaire *et al.*, 2002) and enterotoxin C (Hu *et al.*, 2006; Chang *et al.*, 2008) or other virulence factors as  $\alpha$ -hemolysin (Hla) (Bubeck & Schneewind, 2008) and the Panton-Valentine leukocidin (PVL) (Brown *et al.*, 2009). An intranasal application of conjugated vaccines containing a non-toxic mutant of toxic shock syndrome toxin 1 (TSST-1) and non-toxic heat labile mutant toxin (mLT) of *E. coli* effectively induced an increase in the level of anti-TSST-1 antibodies in the serum and in the nasal secretion. They protected mice from both colonization of the nasal cavity and also from systemic infection (Narita *et al.*, 2008). Two highly immunogenic fragments of enterotoxin B (SEB) induced the production of specific antibodies in chicken. Passive immunization of the rhesus monkeys with these antibodies protected them from death after intranasal application of lethal dose of SEB (LeClaire *et al.*, 2002). In other experiments intranasal immunization with a non-toxic double SEC mutant and cholera toxin (CT) as an adjuvant protected mice from infection (Hu *et al.*, 2006). Bubeck and Schneewind (2008) with the use of a mutant  $\alpha$ -hemolysin (Hla H35L)

which does not form pores obtained specific antibodies in mice which protected them from *S. aureus*-induced pneumonia. Moreover, the anti-Hla antibodies not only protected naive animals from the development of infection but also human lung epithelial cells from damage following infection (Bubeck & Schneewind, 2008). In another study mice with pneumonia that additionally developed skin infection challenged with epidemic community acquired MRSA (CA-MRSA) strain USA300 revealed a high anti-PVL response after immunization with PVL (Panton-Valentine Leukocidin). Depending on the way of immunization, the protection against one of the infections could be obtained. Intranasal vaccination protected from the pulmonary infection but not from the intradermal one, while subcutaneous vaccination protected against the intradermal but not the pulmonary infection (Brown *et al.*, 2009).

Vaccines against *S. aureus*-secreted virulence factors (exotoxins and toxin-like enzymes, Table 2) are suggested to be of limited use as it is hard to determine the group of patients to be vaccinated. Hypothetically, it could concern the personnel of health-care-associated centers with inefficient hygienic procedures like orphanages, nursing centers, prisons especially when increased numbers of infected patients are reported, e.g. patients suffering from pneumonia or PVL+ *S. aureus* cellulitis, or in case of an epidemic of staphylococcal food-borne diseases (SFD).

### 4. Vaccines against other virulence factors

Other targets essential for *S. aureus* virulence have also been examined. The RNAPIII activating protein (RAP), which is important for regulation of virulence, was given to mice and a protective effect against infection was obtained in 72% of them in comparison with 30% in a control group ( $p < 0.0001$ ) (Balaban *et al.*, 1998). MRSA infections are difficult to treat due to the resistance mechanism and the limited therapeutic options. A vaccine directed against the PBP2a (penicillin-binding protein 2a) protein showed potential to improve therapy outcome and long-term prognosis. Senna *et al.* (2003) cloned an internal region from the transpeptidase domain of PBP2a into a mammalian expression vector which was then used as a DNA vaccine for immunization of mice. After three rounds of immunization a significant increase in the level of specific anti-PBP2a antibodies was obtained and the bacterial load in kidneys was zero (in 80% of the examined animals) ( $p < 0.015$ ) indicating the efficacy of immunization.

PBP 2a inhibition-based strategy seems to be a promising approach. Indeed, it would not prevent infections, but rather would extend therapeutic options by restoring the sensitivity of bacteria to  $\beta$ -lactam antibiotics. Following this idea, it would be interesting to evaluate if blocking *mecA* or the *SCC<sub>mec</sub>* (Staphylococcal cassette chromosome *mec*) element blocking would restore the susceptibility to  $\beta$ -lactams or other groups of antibiotics.

### H. Other factors

Fibronectin-binding proteins (FnBP) are responsible for adherence of *S. aureus* to and subsequent internalization by phagocytes. Intramuscular application of a recombinant fragment of the fibronectin-binding domain (rFnBF), which potentially inhibits the entrance of *S. aureus* to host cells, counteracted the formation of abscesses in guinea pigs. The protective effect was dose-dependent and increased the efficacy of preventive treatment if applied simultaneously with cefazolin. The ef-

ficacy of this method relies on the competition in the adhesion of exogenic *S. aureus* rFnBF with FnBP, which causes infection (Menzies *et al.*, 2002).

The use of a global regulation system — accessory gene regulator (*agr*) — to reduce strain virulence, is an example of a new promising therapeutic strategy (Cheung *et al.*, 2004).

It has also been reported that the Autoinducer Protein (AIP) originated from one *S. aureus* strain may either activate or inhibit the expression of RNAIII, which can block the activity of the *agr* regulon in other *S. aureus* strains. It can also potentially be a therapeutic option and deserves further investigations. The bacterial quorum-sensing system could be deregulated with the AIP protein, preventing biofilm production. Nevertheless, it ought to be taken into consideration that modulation of the *agr* regulon can inhibit production of some virulence factors while simultaneously activating the expression of others (Harraghy *et al.*, 2007).

## SUMMARY

The treatment of high risk patients without applying appropriate antibiotic policy in hospitals generates various antibiotic-resistance mechanisms among microorganisms. It limits the use of commonly available antibiotics and antimicrobial drugs. Moreover, the spread of resistant bacterial strains across the world becomes more and more common and threatens enhanced morbidity and mortality. Because of multidrug resistance, especially among HA-MRSA, alternative and effective therapeutic options as well as prophylaxis of microbial infections are badly needed. Some of the compounds and methods still need many further studies, some of them seem to be promising in approaching a clinical use in not too-distant a future.

## Authors' contributions

JK wrote chapters Antibacterial Natural Compounds, Old Medicaments — New Approach, Cationic Antimicrobial Peptides, Bacteriophages, and Staphylococcal Vaccines.

MG wrote chapters Staphylococcal Diseases, Preventing Biofilm Formation, Photodynamic Therapy, and Other Factors.

Both authors read and approved the final manuscript.

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