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Surmounting Antimicrobial Resistance in the Millennium Superbug: *Staphylococcus aureus*

Review Article

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Abstract: *Staphylococcus aureus* is the third most dreaded pathogen posing a severe threat due to its refractory behavior against the current armamentarium of antimicrobial drugs. This is attributed to the evolution of an array of resistance mechanisms responsible for morbidity and mortality globally. Local and international travel has resulted in the movement of drug resistant *S. aureus* clones from hospitals into communities and further into different geographical areas where they have been responsible for epidemic outbreaks. Thus, there is a dire necessity to refrain further cross movement of these multidrug resistant clones across the globe. The plausible alternative to prevent this situation is by thorough implementation of regulatory aspects of sanitation, formulary usage and development of new therapeutic interventions. Various strategies like exploring novel antibacterial targets, high throughput screening of microbes, combinatorial and synthetic chemistry, combinatorial biosynthesis and vaccine development are being extensively sought to overcome multidrug resistant chronic *Staphylococcal* infections. The majority of the antibacterial drugs are of microbial origin and are prone to being resisted. Anti-staphylococcal plant natural products that may provide a new alternative to overcome the refractory *S.aureus* under clinical settings have grossly been unnoticed. The present communication highlights the new chemical entities and therapeutic modalities that are entering the pharmaceutical market or are in the late stages of clinical evaluation to overcome multidrug resistant *Staphylococcal* infections. The review also explores the possibility of immunity and enzyme-based interventions as new therapeutic modalities and highlights the regulatory concerns on the prescription, usage and formulary development in the developed and developing world to keep the new chemical entities and therapeutic modalities viable to overcome antimicrobial resistance in *S. aureus.*

Keywords: *S. aureus • MRSA • Vaccines • MDR • Rational drug design • Combinatorial biosynthesis • Enzymibiotics*

1. Introduction

"*Antimicrobial resistance is a global quandary demanding urgent action.*" Infectious diseases after cardiovascular diseases are the world's leading killers, accounting for approximately 13.3 billion deaths, which are a quarter of the total deaths occurring globally. *Staphylococcus aureus* a gram-positive coccus is a commensal on human skin and mucosa. Approximately 20% of the human population colonizes *S. aureus*, 60% are intermittent carriers and 20% never ever carry the microbe. It is an opportunistic pathogen when it gains access to the body via a cut or an abrasion. *Staphylococcus* is commonly responsible for SSTI's (Skin and Soft Tissue Infections) like boils, carbuncles, abscesses, impetigo [1] and other serious conditions, and it is responsible for bacteraemia, oseteomyelitis, meningitis, pneumonia, and abscesses in the liver, kidney and spleen. Patients who have prosthetic devices or underwent invasive procedures are also prone to staphylococcal infections [2,3]. The presence of resistant *Staphylococcal* isolates both in nosocomial and community settings has prevailed upon scientists to develop drugs for the treatment of infections caused by resistant *staphylococci*. Methicillin a penicillase resistant β-lactam of the penicillin class was introduced in 1959 (Beecham) but *Staphylococcus aureus* rapidly acquired resistance, leading to the emergence of Methicillin-resistant *S.aureus* (MRSA). The very first report of MRSA came from a British Hospital [4]. Later, clones of MRSA spread across international borders, adapted to the new settings and became resident clones, which are responsible for nosocomial infections

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and community epidemics [5]. The presence and spread of MRSA and its variants have created a critical situation for clinicians, as MRSA was more difficult to treat.

1.1. *Staphylococcus aureus* - the Millennium Super bug

Sir Alexander Ogston [6] recognized the human pathogenic potential of *Staphylococcus aureus* in wound suppuration. Thereafter a mortality rate of 82% in 122 patients at the Boston City Hospital suffering with *S. aureus* bacteremia further confirmed its virulence and pathogenicity [7]. MRSA posed further difficulty by acquiring resistance against other classes of antibiotics, the very first being Gentamicin. The prevalence of Gentamicin-resistant MRSA (GR-MRSA) in Australia, USA and Europe was accounted in the early 1980's. GR-MRSA has reduced susceptibility to tetracyclines, erythromycins, lincomycins, novobiocin, neomycin and kanamycin. The majority of surveys have indicated that more than 50% of MRSA isolates are resistant to Macrolides, Lincosamides, Floroquinolones, and Aminoglycosides [8,9]. The United Kingdoms is the worst hit in Europe with *Staphylococcal* infections. Fourty-five percent of the 19,311 infections in 2003-04 were caused by MRSA. More recently, MRSA has exhibited resistance to Ciprofloxacin and Mupirocin [10-13]. Ciprofloxacin was one of the first Fluoroquinolones intensively used for the treatment of MRSA infections [14]. Resistance to ciprofloxacin by MRSA has been reported from clinical specimens in the early 1990's [15]. The maximum number of hospitalizations due to MRSA resistant to fluoroquinolones was recorded between 1999 and 2000 at approximately 125,969 hospitalizations including 31,440 for septicemia, 29,823 for pneumonia and 64,706 for other infections [16].

Glycopeptide antimicrobials, Vancomycin and Teicoplanin have been widely used to control MRSA infections for the last two decades. As a consequence of the selective pressure of Vancomycin, *S. aureus* with reduced susceptibility towards Vancomycin emerged [17] and the first Vancomycin intermediate *Staphylococcus aureus (*VISA) was reported from Japan [18]. However, the first true isolate of *S. aureus* with Vancomycin resistance, i.e. Vancomycin resistant *S.aureus* (VRSA), was reported from Michigan in July 2002 and was followed by a second from Pennsylvania [19,20].

Staphylococcus aureus is a well-armed super pathogen since it possesses extreme genetic plasticity and an array of virulence factors that provide it resistance to different classes of antimicrobial agents. This highly adaptable and virulent gram-positive microbe has established itself as an endemic entity in hospitals and other health care settings and has also started moving into communities, thereby evolving into variants popularly known as Community-Acquired MRSA (CA-MRSA), Epidemic MRSA (E-MRSA), Hospital-Acquired MRSA (HA-MRSA) and Multi-antibiotic resistant *S.aureus* (MARSA). CA-MRSA arise either *de novo* from the community-based *S. aureus* strains, referred to as true CA-MRSA, or through hospitals and health care settings. CA-MRSA was first reported in the early 1990's in people who had no previous history of hospitalization [21-24].

CA-MRSA is of great concern, in particular clone USA 300, which is an epidemic clone in communities in the USA and Europe leading to SSTI's and causing necrosis of soft tissues. It differs from HA-MRSA by the presence of Panton-Valentine Leukocidin (PVL) genes, which express an enzyme responsible for skin infections [24]. PVL is a synergistic membrane-tropic toxin involved in skin or skin structure-related infections in humans. The risk factors of CA-MRSA infection include frequent use of intravenous drugs, prior antibiotic use, presence of diseases like diabetes mellitus, and malignancy [25,26]. CA-MRSA outbreaks have been reported from educational institutes, prisons, naval ships, hospital nursery and maternity units, and among athletes and gays [27-31]. CA-MRSA is resistant to all β-lactam antibiotics and exhibits cross-resistance to other classes of antimicrobial agents [32]. Regardless of origin, the community is increasingly becoming a reservoir for MRSA [33]. CA-MRSA has also been reported as an unusual cause for the lethal community-acquired pneumonia (CAP) occurring as a post-influenza infection. There are reports from USA and Australia of CAP caused by CA-MRSA [34,35]. The CAP-causing CA-MRSA strain reported from Australia is fatal since it leads to death within 48 hours of the infection. Unfortunately, there are limited therapeutic options to combat CAP caused by CA-MRSA [36]. The rapid spread of the epidemics caused by MRSA and its variants (VRSA, HA-MRSA and CA-MRSA) has increased to an extent where it is going to outnumber the cases of HIV-infected individuals in the USA alone within a short time. Thus, the threat posed by these multidrug resistant gram-positive cocci could lead to a global catastrophe of intractable infections and demands development of modalities to restrict MRSA zonally and overcome its resistance on a global basis [37].

2. Survival tactics of *S. aureus*: super or supreme genomic plasticity

MRSA variants pose a threat of reduced susceptibility to practically all antimicrobial drugs including the latest semi-synthetic streptogramins Quinopristin-Dulfopristin, besides Linzeolid and Vancomycin. What is the genetic

make-up of this gram-positive bacterium that provides it with such immense plasticity as to become a dreaded super pathogen today? Probably *Staphylococcus aureus* has a better control or communication strategy within itself to manipulate genetically and acquire tolerance or resistance to any and every kinds of antimicrobial agents or biocides. Antimicrobial drugs generally act at three interfaces in the microbe viz. cell wall synthesis, protein synthesis and nucleic acids.

2.1. Cell wall inhibitors

β-lactams, which launched mankind's journey into the golden era of antibiotics with drug like Penicillin and Methicillin, basically inhibit cell wall synthesis by presenting them as pseudo-substrates for the transpeptidases (now Penicillin-binding proteins or PBP) that acylated them, thereby causing weak peptide bond formation susceptible to osmotic changes and leading to lyses of the bacterium. Five different PBP's viz. 1,2,3,3',4 have been described for susceptible strains of *S. aureus*. A variant, which is responsible for resistance to β-lactams, is PBP2a [38]. PBP2' or PBP2a is a surrogate transpeptidase having low or no binding affinity for β-lactams as compared to other PBP and therefore can express at antibiotic concentrations that are lethal for other PBP, thereby conferring resistance to *S. aureus* [39-41]. Specific Methicillin resistance results in the presence of the *mecA* gene, which is a part of the genomic island designated as *Staphylococcal* cassette chromosome *mec (SCCmec). mecA* is also responsible for synthesis of PBP2a [42,43]. Vancomycin-induced susceptibility through the process of inhibition of transglycosylation and trans-peptidation steps is achieved by blocking the precursor, D-Alanyl-D-Alanine (D-Ala-D-Ala), by the antibiotic through hydrogen bonding thus inhibiting the formation of UDP-MurNAc-pentapeptide required for cell wall synthesis. The Vancomycin-resistant microbes alter the precursor from D-Ala-D-Ala to D-Ala-D-Lac using the enzymes Van H dehydrogenase and Van A ligase thereby making the normal binding site unavailable. Furthermore, they release free D-Ala-D-Ala to bind to vancomycin and accumulate the cell wall [44,45].

2.2. Inhibitors of bacterial translation

The difference between the prokaryotic and eukaryotic protein synthesis machinery has been instrumental in designing and discovering antibiotics. Thus, inhibiting protein synthesis serves as a multidimensional drug target involving steps of initiation, elongation and termination for designing the protein assembly to induce susceptibility. Furthermore, there could be other minor processes that could also be interceded by the use of inhibitors.

Macrolides, Aminoglycosides, Tetracyclines and Oxazolidinones are the major drug classes that function via inhibition of protein synthesis. Macrolides are variable-sized lactones attached to one more amino or neutral sugars. Clarithromycin, Erythromycin and Roxrithomycin are 14-member lactone ring macrolides while Azithromycin is the only macrolide having a 15-member lactone ring. Bacteria resort to three methods to resist macrolides: (a) target site modification by methylation or mutation thereby preventing binding of the antibiotic to the ribosomal target; (b) efflux of the antibiotic; and (c) inactivation of the antibiotic. The *erm* gene is responsible for resistance of *S. aureus* to erythromycin by expression of *erm* methylase, which dimethylates the single adenine residue in the nascent 23S rRNA, a part of the 50S ribosomal subunit. Approximately 40 *erm* genes have been reported so far [46]. Aminoglycosides, despite being an important anti-staphylococcal drug class, have lost sensitivity to MRSA by deactivation of their structure at particular amino groups thus losing binding affinity to the 30S ribosomal subunit. The major enzymes that participate in this mechanism are ANT (Adenylate transferase) and AAC (Acetyl transferase). Resistance to neomycin, kanamycin, tobramycin and Amikacin in *Staphylococci* are mediated by an ANT(4') enzyme. Macrolide resistance among gram-positive microorganisms is also attributed to two types of pumps, the ATP-binding cassette (ABC transporter) family and the major facilitator superfamily (MFS). In the *Staphylococcus* species the resistance is acquired through the ABC transporter encoded by plasmid-borne *msr (A)* genes [47]. Tetracyclines are broad-spectrum antibiotics, which find use as the second line of treatment for bacterial infections after penicillin. Frequent use of tetracyclines, with its capacities to promote growth in animal husbandry and to treat infections in plants, has led to widespread resistance in diverse bacteria possessing wide ranges of resistant determinants [48]. MRSA acquire tetracycline resistance through either efflux of the drug due to acquisition of *tetK* and *tetL* genes on the plasmid or through ribosomal protection mediated through *tetM* or *tetO* determinants located on the transposon or the chromosome [49,50]. Further studies on the distribution of tetracycline resistance using molecular tools revealed that *tetM* is the major determinant of resistance in MRSA while *tetK* is prevalent in MSSA isolates [51,52]. Linezolid is an oxazolidinone antimicrobial agent registered by DuPont (1980) as an antibacterial drug with steady inhibitory action by blocking peptidyl tRNA binding at the P-site of the 50S ribosomal unit and preventing the formation of the 70S initiation complex [53]. Despite being a synthetic moiety with no history of preexisting resistance mechanism,

Linezolid encountered resistance unexpectedly in *S. aureus*. The reduced Linezolid susceptibility in *S. aureus* [54] occurs through specific point mutations in domain V of 23S ribosomal RNA of the 50S subunit. Domain V represents the peptidyl transferase, which catalyzes the peptide bond formation [55]. Quinupristin-Dulfopristin (QD) inhibits protein synthesis in *S. aureus* by binding to the 50S ribosomal unit. However, reduced susceptibility to the QD combination in *S. aureus* has been noticed recently [56].

Another mechanism to resist antibiotics is via drug efflux proteins or transporters also known as efflux pumps. The prokaryotic kingdom possesses five different kinds of efflux pumps, namely MATE (multidrug and toxic efflux), MF (Major facilitator), SMR (small multidrug resistance), RND (resistance-nodulation-division) and ABC (ATP-binding cassette). Efflux pumps export an extensive range of structurally unrelated antibiotics from the cell, resulting in reduced intracellular concentration of the biocide and thus reduced susceptibility. The best characterized MDR pumps are MFS proteins Qac A/B and Nor A. Qac A expels approximately 30 chemical compounds belonging to 12 chemical classes that could be broadly classified as toxic mono/bivalent ions, cations, and lipophilic compounds, whereas Nor A is related to fluoroquinolones [57,58]. Two efflux pumps (ABC transporters) vgaA and vgbB also play important roles in reduced sensitivity to streptogramins [59].

2.3. DNA damage or Mutation

Linezolid resistance in *Staphylococcus aureus* is due to mutation in gene encoding for riboprotein L4. *S. aureus* exploits the same mechanism for macrolide resistance [60]. The general mechanism of Fluoroquinolone resistance in *S. aureus* functions either by bringing a change in the chromosome responsible for the expression of topoisomerase IV or DNA gyrase or by inducing a multidrug resistance efflux pump. The first resistance mechanism is alteration of the *gyr* A gene that encodes subunit A of DNA gyrase, i.e. topoisomerase, the target of fluoroquinolones. Another mechanism to attain fluoroquinolone resistance relates to mutation in the *Sma*I A locus of the chromosomal DNA of *S. aureus* [61]. Amino acid substitutions in vital regions of the enzyme-DNA complex commonly referred as the quinolone resistance-determining region (QRDR) reduces affinity of quinolones for both of its targets [62]. The reduced susceptibility of MRSA to the Quinupristin-Dulfopristin (QD) combination is due to genes coding for acetyltransferases *vatA*, *vatB* and *vatC* [63].

3. Strategies to combat multidrug resistant *S. aureus*

The major challenge faced by clinicians, para-medical personnel and health care workers is checking the spread of MRSA from infected individuals in health care settings into the community. Prime general strategies for preventing antimicrobial drug resistance are to strictly adhere to guidelines of antibiotic administration, to avoid inadequate treatment, and to restrict the use of certain antibiotics or antibiotic classes from hospital formulary [64,65]. Using old antibiotics with narrow spectrums could possibly be another strategy to overcome resistance, or a combination of old and new antimicrobial drugs could possibly give better control over drug-resistant microbes. Spread of MRSA through horizontal transmission is an important measure to check MRSA infections. This is achieved through prevention of colonization of resistant *Staphylococci* in the nasal and moist regions of the body. Handwashing is the most common practice of bacterial decolonization in health care workers [66]. Body washing with topical antibiotics like Mupirocin lotions with combinations of systemic antibiotics could serve as a regimen to check the colonization and spread of MRSA [67]. The above strategies however do not provide a complete solution to the prevailing problem of acquisition of drug resistance in *Staphylococcus aureus*. The other strategies, which are under investigation by scientists, include:

- Expansion of the known drug classes
- Rational drug discovery – search for new antimicrobial drug targets
- Development of novel formulations of existing drugs
- New chemical templates
- Immunotherapeutics

3.1. Expansion of the known drug classes

Combinatorial chemistry has served the purpose of generating new congeners from the existing chemical structures. However, the chances of them being resisted is on higher side due to their indiscriminate use and also because of their similarity to the templates from which they have been derived that have already become resistant (Figure 1).

4. β - LACTAMS

4.1. Ceftobiprole

BAL5788, now designated as Ceftobiprole, is a redesigned β-lactam group of antibiotics that has potential anti-MRSA activity (MIC range≤0.06–4.0mg/L),

developed jointly by Basilea Pharmaceuticals and Cilag GmbH, a Johnson & Johnson International Company. The compound has potential activity against Linezolid and Vancomycin resistant *S. aureus* (VRSA) [68]. It had completed the phase III first comparative trial in November 2006.

4.2. CAB-175

Another subclass of Cephalosporin is CAB-175, a parenteral cephalosporin of the azeomethine sub-class developed by Cubist Pharmaceuticals. The MIC_{90} of this compound is 4µg/ml. Another anti-MRSA parenteral carbapenam in phase one clinical trials is CS-023 (RO4908643), developed by Roche under license from Sankyo. The compound is active against both MRSA and Methicillin resistant *S. epidermidis*. CS-023 had recently completed Phase I clinical trials [69].

4.3. Tetracyclines

Glycylcyclines (N,N-dimethylglycylamido-9-aminominocycline (DMG-MINO) and DMG-DMDOT), a new class of tetracyclines have been synthesized, which has a potent broad spectrum antibacterial activity. Glycylcyclines inhibit both tetracycline-resistant and sensitive fractions exhibiting ribosomal protection factors i.e. *tetM* and *tetO* or efflux determinants *Tet A*, *E L* and *K* [70]. The MIC_{90} of [(dimethylamino)glycylamido]-6-desmethyl-6-deoxytetracycline (DMG-DMOT) against MRSA was 2µg/ml whereas minocycline had a value of 16 µg/ml. The MRSA exhibiting *TetK* determinant was also susceptible to DMG-DMDOT [71]. DMG-MINO and DMG-DMDOT are more active than vancomycin, erythromycin and ciprofloxacin against both MRSA and MSSA.

4.4. Tigecycline

Tigecycline is a semi-synthetic derivative of DMG-MINO introduced by Wyeth after clearance from the U.S. FDA in July 2005. It is the first glycylcycline, introduced 30 years after the discovery of Minocycline [72]. Tigecycline is stable against all the previously known tetracycline resistant mechanisms and has potential activity against infections caused by MRSA, VRSA and MSSA. It has a MIC for MRSA in the range of 0.25-0.5 µg/ml. It exerts its antimicrobial action by blocking the movement of aminoacyl tRNA into the A site of the 30S ribosome, thus completely blocking the process of translation [73,74]. To date, it has been impossible to generate Tigecycline-resistant isolates under laboratory conditions using suboptimal concentration exposure technique.

4.5. PTK-0796

More recently, another member of the tetracycline class that is under phase 1 clinical trial is PTK-0796, a novel broad spectrum aminomethylcycline family, which is being tested by oral as well as intravenous routes to combat MRSA. In animal models of systemic *S. aureus* infection, PTK-0796 performed better or equal to currently marketed therapeutic drugs and overcame tetracycline resistance in *S. aureus* [75]. Merck Co. & Paratek Pharmaceuticals have entered into collaboration for joint development of this molecule [76].

4.6. Glycopeptides

Higher versions of glycopeptides designed to overcome MRSA and VRSA strains, resistant to Vancomycin and Tecioplannin, include Oritavancin and Dalbavanacin.

4.7. Oritavancin

Formerly known as LY333328, Oritavancin was discovered by Eli Lilly based on structure-activity relationships (SAR) and is currently being developed by InterMune Ltd. (USA). It is the 4'-chlorobiphenylmethyl derivative of chloroeremomycin, an analogue of Vancomycin, possessing potential activity against MSSA, MRSA, VRSA and GISA with a MIC range of 0.03 to 8µg/µL and good tolerance as indicated by phase I clinical studies [77,78].

4.8. Dalbavancin (BI397)

Dalbavancin is a lipoglycopeptide derivative of Teicoplanin, originally discovered as a natural glycopeptide A40926 by Bioresearch Italia and then licensed to Versicor. Both of these companies have undergone a merger into Vicuron Pharmaceuticals, which filed the new drug application in 2005 and was subsequently acquired by Pfizer (USA). Dalbavancin is more potent than Oritavanacin and has a MIC range of 0.03-1 µg/µL for MRSA, MSSA, VRSA and GISA [79,80]. Pfizer received approval from the U.S. FDA for Dalbavancin hydrochloride in December 2007.

4.9. Ramoplanin

Oscient Pharmaceuticals and Vicuron Pharmaceuticals have developed a glycolipodepsipeptide antibiotic, which is potentially active against multidrug-resistant *Staphylococcus aureus* [81]. Currently it is in Phase III clinical trial for the treatment of Vancomycin-resistant enterococci and *Clostridium difficale*-associated diarrhea (CADD) [82]. No cases of laboratory generated or clinical resistance have been reported to date.

5. Fluoroquinolones

5.1. Garenoxacin

Garenoxacin was under investigation as T-3811ME, a novel des-F(6) Quinolone that has very potent anti-MRSA activity when compared to other members of the fluoroquinolone family [83]. It overcomes the GyrA and ParC (GrlA) mutations involved in providing *S. aureus* with resistance to fluoroquinolones. The MIC_{90} range for MRSA is 0.025-0.625 μg/ml [84]. It has been rechristened as Garenoxacin and Toyama Chemical Co. (Tokyo) and Schering Plough Corp. (USA) have jointly filed an NDA in 2005.

5.2. Sitafloxacin

Sitafloxacin, initially investigated as DU-6859A and then designated as Gracevit™ (Daiichi Pharmaceuticals), is approximately four times more potent than other fluoroquinolones [85]. The MIC of Sitafloxacin against MRSA and ciprofloxacin-resistant isolates ranges between 0.05 and 0.5 mg/L. This has successfully cleared phase III clinical trials in Japan.

5.3. WCK-771

Wockhardt (India) has synthesized the broad-spectrum fluoroquinolone WCK-771, which has potential activity against MRSA as well as fluoroquinolone-resistant *Staphylococci*. The potency of WCK-771 is four to eight fold higher than other fluoroquinolones in combating VISA and VRSA. The Nor A efflux pump did not have any activity against WCK-771 [86]. This arginine salt of S-(−)-nadifloxacin is under phase II clinical trials.

6. Oxazolindinones

6.1. Ranbezolid

Ranbezolid (RBx-7644) is a new extended spectrum oxazolidinone class of compound, discovered by structure-activity relationship (SAR) studies from Ranbaxy Research Labs, India [87]. The compound possesses some excellent features of Linezolid and is effective against all anaerobes. RBx-7644 is also effective against biofilms formed by MRSA [88]. Phase I clinical studies have indicated that Ranbezolid is well tolerated and safe [89,90].

6.2. AM-7359

Another oxazolidinone class of compounds under development is AM-7359 from Merck. It is eight-fold more efficacious than Linezolid against MRSA in a mouse infection model [91].

6.3. Rational drug discovery

Rationality refers to using a defined strategy for antimicrobial drug development based on the knowledge of genome structure (genomics) or protein expression profiles (proteomics).

A good target generally is a gene product or an enzyme having a wide occurrence in clinical isolates. Bacterial genomics have taken great strides and today over a dozen bacterial pathogens including *Staphylococcus aureus* have been completely sequenced for their genomic organization. This has led to further evaluation of the identity of chemical molecules or plant extracts referred as “hits,” which inhibit the expression of certain sections of genome ascribed to virulence and multidrug resistance.

def and *fmt* genes were initially discovered in *E. coli* for their putative role in formylation of methionine to initiate protein synthesis, and subsequently the N-formyl group was removed from the nascent polypeptide by the enzyme peptidyl deformylase. Genomics has indicated that bacteria have at least one homolog of the *def* gene coding for active deformylase (a matrix metalloproteinase) [92]. LBM 415 (NVP PDF-713), an investigational new peptide deformylase-inhibiting compound from Novartis, has a MIC_{90} of 1mg/ml against oxacillin-resistant *Staphylococcus aureus* [93].

The Fatty Acid System (FAS) that governs the fatty acid synthesis in bacteria as well as in humans is found to be structurally and functionally different through genomic studies. Human FAS system is a single multidomain complex whereas in bacteria, plants and protozoans there are distinct components. Further sequencing has revealed that overall sequence homology between the two FAS component systems does not exist despite similarity in structure and function. Based on these studies, bacterial fatty acid synthesis is considered as an efficient antimicrobial drug target [94] Diazoborines like Thiolactomycin and Cerulenin kill bacteria by inhibiting FabI (enoyl-ACP reductase) but have limited therapeutic use due to lack of inherent toxicity. Triclosan is another FabI inhibitor [95]. Experimental evidence exists that FabI and the fatty acid initiation, chain elongation and condensing enzymes play important roles as targets for bacterial fatty acid synthesis inhibition. GlaxoSmithKline has identified two hits from their high throughput-screening program of compound libraries belonging to the benzidiazapine and imidazole series. The imidazole series lead induced 16-fold improvement in antibacterial activity and five-fold improvement in the potency of FabI inhibition [96].

Knockout mutants of *S. aureus* provide information on selective genes that play an important role in

Figure 1. New extended class of antimicrobial drugs under different phases of clinical trials.

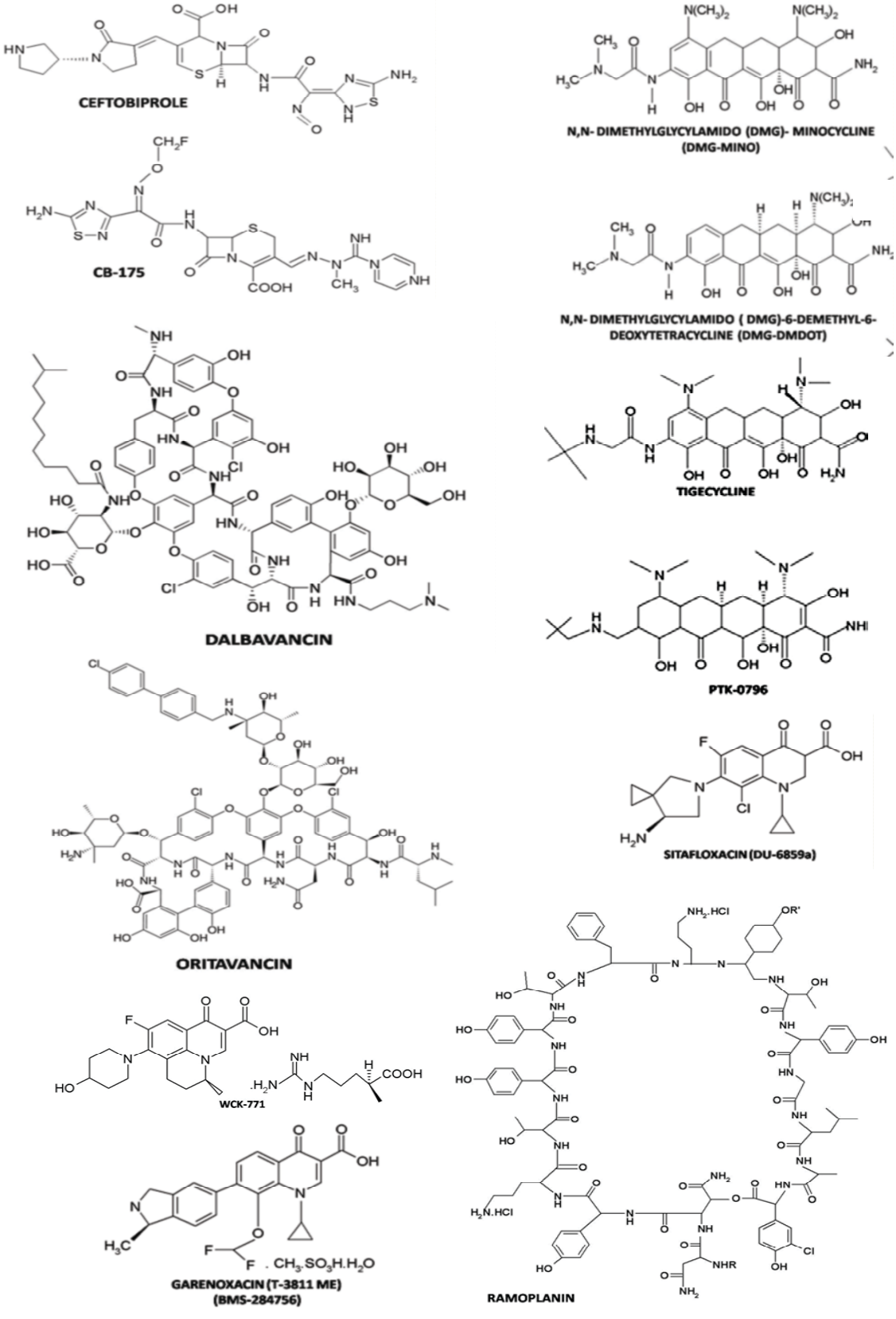
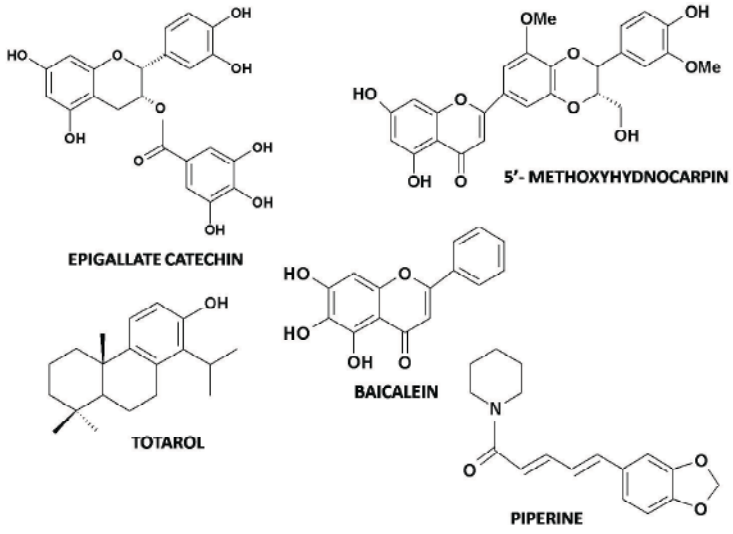


Figure 2. Promising antimicrobial drug resistance reversal agents (biopotentiators).



the survival and infection. The sortase (Srt) gene, responsible for virulence induction on the capsular polysaccharide, could serve as a potential avirulence target in designing new anti-*staphylococcal* drugs. Gene knockout studies have indicated the role of the SrtA gene in pathogenesis by modulating the affinity of cell surface proteins and adhesions to host cells and thereby their virulence [97]. Mutant *S. aureus* was not able to induce infections without affecting the viability. Some promising candidates exhibiting irreversible inhibition of sortase include β-sitosterol-3-O-glucopyranoside from *Fritillaria verticillata*, and berberine chloride from *Coptis chinensis* exhibited potent SrtA [98]. Para-aminobenzoic acid (PABA) has also been found to be a good target for the development of new antimicrobials, as it is a precursor of folic acid, which is used for the synthesis of vitamins by bacteria and protozoans [99]. The Shikimate pathway for the synthesis of aromatic amino acids is another potential target for antibacterial drug design since both PABA and the Shikimic acid pathway are absent in the human system. Two-dimensional gel electrophoresis interfaced with mass spectroscopy has become a boon in understanding the expression of microbial systems under stressful conditions and has a significant role in identifying targets around which new structures could be woven to design potential antibacterial drugs [100].

Transcription profiling methods like Oligonucleotide arrays have been successful in the identification of virulence as well as oxacillin-resistance genes in *S. aureus* [101]. Phage-mediated bacterial growth inhibition could also be exploited for developing new drugs to combat the problem of antimicrobial drug resistance. With help from the antibacterial activity of phage open reading frames (ORF's), new proteins would serve as targets for the development of new small molecule inhibitors of proteins having high susceptibility towards *Staphylococcus aureus* [102].

6.4. Development of Novel formulations from existing drugs

The new drugs discovered through combinatorial chemistry and rational drug design, i.e. driven by genomics and identifying novel drug targets, should be conserved and utilized only under chronic and serious infections fatal to life. There is a need to re-potentiate the current armamentarium of drugs to overcome the resistance posed by MRSA clones. Antibiotic efflux mechanisms and inhibitors of bacterial enzymes modifying the antimicrobial drugs can serve as formulations with enzymes or MDR pump inhibitors to potentiate these resistant antibiotics. The very first natural β–lactamase inhibitor was clavulanic acid, isolated from *Streptomyces clavuligerus,* that competitively and irreversibly inhibits β-lactamase. β-lactamase-sensitive drugs formulated with clavulanic acid overcame resistance in MRSA. The first combination developed was AUGMENTIN® (combination of semi-synthetic penicillin Amoxycillin trihydrate and the postassium salt of Clavulanic acid) used against β-lactamase-producing *S. aureus* by GlaxoSmithKline (GSK). MRSA and MSSA endocarditis animal models and clinical trials exhibit effective control by AUGMENTIN® [103]. AUGMENTIN (Co-Amoxiclav) was efficacious like vancomycin in the control of MRSA as well as non-lactamase isolates of *S. aureus* [104]. More recently imipenam/cefazolin, vancomycin and netilmicin, a triple antibiotic combination, is very effective for the treatment of MRSA [105]. HA-MRSA is more susceptible to a synergistic combination of Vancomycin

and Gentamycin [106]. The combination of Daptomycin with β-lactams antibiotics like ampicillin-sublactam and ampicillin is synergistic for the possible treatment of MRSA [107]. Cefoperazone sodium and sulbactam sodium combination (1:1) has completed the Phase IV clinical trial sponsored by Pfizer Inc., and is marketed as Magnex for the treatment of intra-abdominal infections including those from β-lactamase as well as non-β-lactamse-producing *S. aureus*.

Plant extracts are being screened for their drug resistance reverting or modifying effects, as they could be putative sources of lead structures, which could render the reuse of redundant drugs encountered due to efflux pumps present in multidrug-resistant infectious microbes. This has revived plant natural products research in search of plant extracts or compounds that have the capacity to revert the resistance of currently used drugs. These are known as resistance modifiers or biopotentiators (Figure 2).

Epicatechin gallate (ECg), initially discovered as compound-P from green tea, has shown to revert the resistance of MRSA towards β–lactams like Oxacillin, Ampicillin and Cefalexin. The mechanism found to enhance the antimicrobial potential of the β–lactams was integrating with the cell wall peptidoglycan thus interfering with the cell wall integrity of the organism [109-112]. Totarol, a dipterpenoid enhanced the activity of methicillin 256-fold against MRSA. It is a respiratory blocker and inhibits respiration by 70% when used singly and when combined with methicillin-inhibited PBP2a expression [113]. Very recently totarol has found to potentiate Norfloxacin by inhibiting the expression of norfloxacin efflux pumps [114].

Reserpine, an anti-hypertensive alkaloid from *Rawolfia serpentina,* is also a Nor A efflux pump Inhibitor (EPI) and invigorates fluoroquinolones like Moxifloxacin, Ciprofoxacin and Sparfloxacin by decreasing their MIC four-fold in resistant *S. aureus* [115]. It also reverses the tetracycline resistance by inhibiting the Tet(k) efflux protein [116]. Berberis plants, apart from having the putative antimicrobial-berberine, also possess multidrug pump inhibitor-inhibitors 5-methoxyhydnocarpin D (5-MHC-D) and pheophorbide A, which facilitate the penetration of berberine into *S. aureus* [117,118]. 5-MHC-D is also a synergist to Nor A substrates and thus help in potentiating the fluoroquinolones.

Carnosic acid and Carnosol are abietane diterpenoids from *Rosamrinus officinalis* (Rosemary), which enhance the susceptibility of *S. aureus* possessing Tet(K) and MsrA-resistant efflux pumps. Carnosic acid is a Nor A EPI [119]. Baicalein is a trihydroxyflavone extract of Thymus (*Thymus vulgaris*), which exhibits a strong synergistic activity with tetracycline and β-lactams like Oxacillin, Cefmetazole and ampicillin against MRSA [120]. Piperine, an alkaloid of *Piper longum* and *Piper nigrum,* enhances Ciprofloxacin activity against MRSA by reducing the MIC 2-fold by mimicking reserpine as a Nor A EPI [121]. Plant extracts also inhibit virulence factors or enterotoxins A/B and toxins in *S. aureus* thereby reducing the chance of infection [122]. Chloroform extract of *Securinega virosa* (Euphorbiaceae) and ethanol extract of *M. benthamianum* (Caeselpiniceae) potentiated the activity of Fluoroquinolones, tetracycline and erythromycin in resistant *S. aureus* isolates and reduced their MIC four-fold [123]. *Puncia grantum* (pomegranate) methanol extract potentiated the activity of Chloramphenicol, gentamicin, ampicillin, tetracycline and oxacillin against clinical MRSA [124]. Chloroform and ethyl acetate extracts of *Cordiaa gilletii* revived sensitivity of Penicillin and Streptomycin against MRSA [125].

Non-antibiotics as well as synthetic compound libraries have also been evaluated for their antimicrobial drug resistance reversal capacities. Over 9600 compounds have been screened for their Nor A inhibition activity for development of candidate EPI's to potentiate fluoroquinolones against MRSA [126]. Clinically Thioxanthine and Phenothiazine derivatives are antiemetic and neuroleptic agents and have recently been found to exhibit broad antimicrobial activity [127,128]. At sub-inhibitory concentrations, these compounds have synergistic effects against drug-resistant *S. aureus* and several other species of resistant bacteria [129] and have recently been found to inhibit the MDR pump in *S. aureus* [130].

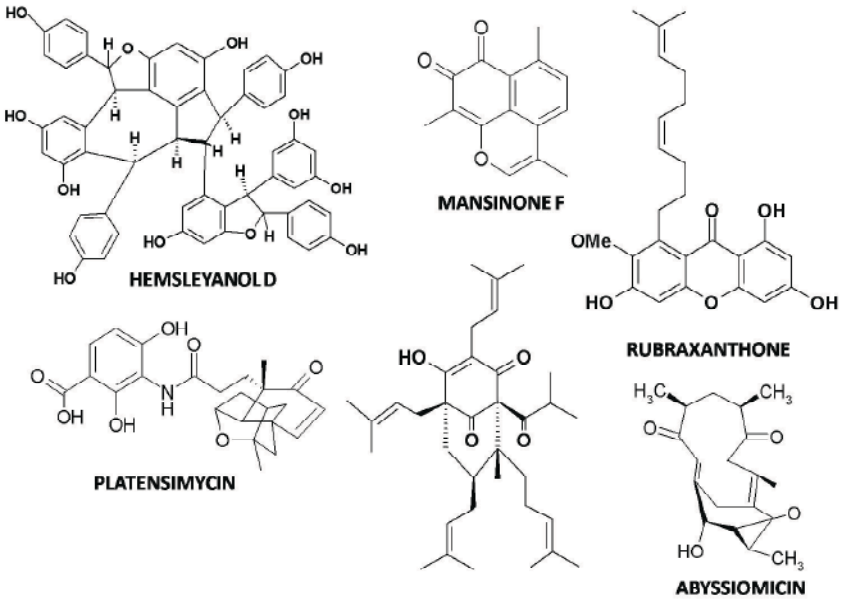
Although several plant extracts and lead compounds have been identified in both academic and industrial research programs as EPI, none of them have undergone clinical evaluation as a possible regimen in chemosensitization of current antibiotics to treat MRSA as an interim modality.

6.5. New Chemical templates

Natural products from microbes have been the mainstay of the pharmaceutical industry for development of new drugs directly or indirectly, as they provide unmatched diversity of chemical templates with potent biological activity. However, plant natural products also provide immense chemical diversity for antimicrobial drug development (Figure 3). Semi-synthesis of natural product analogs developed through combinatorial chemistry provides better chances of a "hit" identification for antimicrobial drug development compared to *de novo* synthesis of compounds [131].

Anti-MRSA activity of plant extracts has been a popular assay in the quest for new anti-*staphylococcal*

Figure 3. Promising plant and microbial natural products for use as anti- MRSA antibiotics.



drugs. Extracts of *Sutherlandia frutescens*, *Callistemon rigidus, Shorea hemslyana, Garcinia spp., Terminalia avicinnoides,* and *Ocimum gratissisimum* have exhibited potential anti-MRSA activity and are thus candidates for exploration of bioactive compounds to overcome resistance in *S. aureus* [132-135]. Some lead compounds from plant extracts have already been isolated and tested for their potential for development of anti-MRSA drugs. Mansinone F, a sesquiterpene from *Ulmus davidiana* var. *japonica,* has potential anti-MRSA activity with a MIC range of 0.39-3.13µg/ml [136]. Hyperforin, acylphlorgucinol from *Hypericum perforatum* (St. John's wort), has a MIC range of 0.1-1 µg/ml against MRSA and PRSP [137]. Hemsleyanol D (stilbene) from *Shorea hemsleyanol* has a MIC of 2µg/ml for MRSA [135]. Rubraxanthone from *Garcinia dioica* exhibited a MIC of 0.313-1.25 µg/ml [138].

Microbial natural products have been the mainstay since the golden age of antibiotics and continue to move ahead [139]. Screening microbes from diverse geographical regions could offer novel chemical diversity for antimicrobial drug development since the majority of antibiotics isolated from microbial systems are endemic due to their occurrence in the temperate region [140,141]. Daptomycin, dalbavancin, and oritavanacin that have been developed to combat MRSA are of microbial origin. Platensimycin from *Streptomyces platensis*, a South African isolate screened by Merck, exhibits potential anti-MRSA activity by inhibiting fatty acid biosynthesis. The MIC range of Platensimycin is 0.5-1µg/ml [142,143]. Abyssomicin is yet another microbial natural product from marine streptomycete *Verrucosispora* Strain AB-18-032 which has potential anti-MRSA activity via inhibition of para-aminobenzoic acid (PABA) synthase, essential for the synthesis of folic acid [99]. Microbes from marine organisms and plant endophytes also serve as underexplored niches for screening new antimicrobial drugs with unique modes of action [144,145]. Munumbicin B from endophytic *Streptomycete* NRRL3052 residing in *Kennedia nigriscans* (snakevine), a medicinal plant, has a MIC of 2.5µg/ml against MRSA [146]. Microbes continue to be novel sources of antibiotics when they are screened strategically using knowledge of biodiversity by employing genomic and proteomic techniques.

6.6. Immunotherapeutics

Development of a candidate vaccine to combat resistance in MRSA has been one of the foremost targets to reduce the burden of chronic *staphylococcal* diseases. *S. aureus* is a difficult bug for vaccine development since it has numerous virulence factors to evade host defence mechanisms. *S. aureus* produces a MHC class II analogous protein (Map), which modulates the T-Cell response thereby potentiating its survival by modulating the host cell immunity [147]. Currently several immunogenic substances are being designed to overcome MRSA through host immunity. Tefibazumab® is a monoclonal humanized MSCRAMM (microbial surface components recognizing adhesive matrix molecules) based vaccine in Phase II clinical trials. StaphVAX® a polysaccharide conjugate vaccine from Nabi Biopharmaceuticals did not clear phase III clinical trials. Its development was terminated due to the requirement of booster doses every 6 months

for protection against *staphylococcal* bacteremia in hemodialysed patients [148]. Another candidate vaccine under study is Aurograb®, a recombinant *S. aureus* vaccine that recognizes GrfA an ATP-binding cassette transporter protein, developed by NuTecPharma [149]. Currently Aurograb® is in phase III clinical trials. Medimmune is undertaking Phase II clinical trials of the antilipoteichoic acid chimeric monoclonal antibody BSYX-A110.

These immune interventions if developed may not be effective enough to counter MRSA infections as monotherapies, hence their compatibility with antimicrobial drugs is reasonable and should be used as a modality to overcome resistance. Along the same line, Aurograb® and Vancomycin have been found to have a predominant synergistic effect for treatment of MRSA infections (Clinical trial no.NCT00217841). Thus, the possibility exists for developing immunogenic interventions and formulating them with antimicrobials.

7. Other strategies

7.1. Enzymibiotics

Lysostaphin is an enzyme (glycylglycine endopeptidase) produced from *Staphylococcus simulans* that has an antimicrobial action on *Staphylococcus aureus* [150]. The renewed interest in using lysostaphin as a modality for treatment of MRSA infections resulted from the decreased effectiveness of current drugs and demand for immediate infection control. Lysostaphin has a maximum bactericidal concentration of 0.16-2.5µg/ml against MRSA [151]. Lysostaphin-based products have been developed for clinical trials in the USA by Biosynexus Inc. [152]. Clinical antibiotic-resistant *S. aureus* exhibited sensitivity to recombinant lysostaphin, highlighting the pre-clinical efficacy of the enzyme as an antibiotic [153]. β–lactam antibiotics have also exhibited potential synergistic action with Lysostaphin in *in vitro* and animal model studies indicating its pivotal role in rejuvenating the current drugs [154]. However its combination with vancomycin is not clear and VRSA and GISA exhibit resistance to Lysostaphin [155]. Though Lysostaphin resistance has been encountered in *S. aureus* due to mutation of the *fem A* gene resulting in altered site having monoglycine cross bridges, the MRSA phenotype is lost [156,157]. Furthermore, it has been found that the reduced fitness cost in Lysostaphin-resistant MRSA induces reduction in virulence thus making them sensitive to β-lactam antibiotics [158]. Thus it could be concluded that Lysostaphin, individually as well as in combination with selected antibiotics, could be effectively used for treatment of MRSA infections.

7.2. Combinatorial Biosynthesis

Microbial systems possess machinery by virtue of which they produce bioactive compounds like antibiotics broadly comprising of amino acids and carboxylic acids, which are oriented in defined complexity spatially and functionally. This machinery constitutes a cluster of genes, which are responsible for the production of giant assembly proteins known as Non-ribosomal peptide synthetases (NPRS) and polyketide synthetases (PKS). The idea of combinatorial biosynthesis came by re-sequencing these clusters of genes at the DNA level [159]. A variety of polyketide drugs like Amphotericin B, Nystatin, Lovastatin, and FK 506 are currently used by the pharmaceutical industry [160]. Production of new polyketides is probably the most advanced application of combinatorial biosynthesis. Genetically altering biosynthesis pathways can potentially generate an inexhaustible number of new polyketides for drug assays and further development. Jacobson and Khosla [161] have developed a technique of chemobiosynthesis, by altering PKS genes in order to make new polyketides. The chemical templates obtained through chemobiosynthesis were superior versions of erythromycin. Expression systems, designed for harvesting new molecules from plants and microbes, are being developed for production of new chemical templates with no previous history of compounds to have co-evolved with the multidrug-resistant microbes to overcome resistance [162,163]. Analogs of Daptomycin (Cubicin®) generated by combinatorial biosynthesis are under evaluation for their potential to combat MRSA [163]. Ramoplanin is the only analogue of 17AA lipodepsipeptide Enduracidin from the soil bacterium *Streptomyces fungicidicus* ATCC 21013. The biosynthesis mechanism includes a 84kb gene cluster containing 25 open reading frames located within a 116kb genetic locus [164].

8. Conclusions

Staphylococcus aureus poses a serious challenge today as it has achieved the status of the most versatile and dreaded pathogen responsible for a variety of human infectious diseases. The alarming situation posed by *S. aureus* demands prevention from further spread and this can be achieved by following the strict recommendations and guidelines for infection control as provided by the CDC/WHO. Strict surveillance of resistance patterns at both nosocomial and community levels using rapid diagnostic methods and genotyping would provide reliable patterns of antibiotic usage. Decolonizing MRSA in health care workers, rotating antibiotics, and curbing indiscriminate

use of antibiotics by strict regulatory guidelines are other measures to restrict further development of antimicrobial drug-resistance and to combat the menace of MRSA. Therapeutic measures need to be designed using tools like genomics and proteomics to help in discovering newer compounds from the biodiversity and in developing new formulations (inclusive of herbals) to overcome the multiantibiotic-resistant *S. aureus*.

References

[1] Peacock S.J.,de Silva I.,Lowy F.D.,What determines the nasal carriage of Staphylococcus aureus?, Trends Microbiol., 2001, 9, 605-10

[2] Pettit C.A., Fowler V.G., Staphylococcus aureus bacteremia and endocarditis,Cardiology Clinics, 2003, 21(2), 219-33

[3] Fang G., Keys T.F., Gentry L.O., Harris A.A.,Rivera N., Getz K. et al., Prosthetic valve endocarditis resulting from nosocomial bacteremia. A prospective, multicenter study, Ann. Intern. Med.,1993, 119(7), 560-67

[4] Jevons,M.P.,Celbenin-resistant staphylococci.,Br. Med. J.,1961,1,124–25

[5] Parker M.T., Hewitt J.H., Methicillin resistance in Staphylococcus aureus, Lancet,1970,1, 800-4

[6] Ogston A., Micrococcus poisoning., J. Anal. Physiol.,1883,17 24–58

[7] Skinner D., Keefer C.S., Significance of bacteremia caused by Staphylococcus aureus., Arch. Intern. Med.,1941,68, 851–75

[8] Archer G.L., Scott G., Conjugative transfer genes in Staphylococcal isolates from the United States, Antimicrob. Agents Chemother.,1991, 33, 2500-04

[9] Trucksis M., Hooper D.C., Wolfson J.S., Emerging resistance to Fluoroquinolones in Staphylococci, Ann. Intern. Med., 1991, 114, 424-26

[10] Lacey R.W., Mitchell A.A.B., Gentamicin-resistant Staphylococcus aureus, Lancet, 1969, II, 1425–26

[11] Lowy F.D., Staphylococcus aureus infections, N. Engl.J. Med.,1998, 339, 520-32

[12] Moellering R.C. Jr., Problems with antimicrobial resistance in gram-positive cocci, Clin. Infect. Dis., 1998, 26,1177-8

[13] Lelievre H.,Lina G., Jones M.E.,Olive C., Forey F.,Roussel-Delvallez M., et al., Emergence and Spread in French Hospitals of Methicillin-Resistant Staphylococcus aureus with Increasing Susceptibility to Gentamicin and Other Antibiotics. J. Clin. Microbiol.,1999, 11, 3452-57

[14] Mulligan M.E., Ruane P.J., Johnston L., Wong P., Wheelock J.P., MacDonald K.,et al. Ciprofloxacin for eradication of methicillin-resistant Staphylococcus aureus colonization. Am. J. Med.,1987,82(4A), 215-9

[15] Harnett N., Brown S., Krishnan C., Emergence of Quinolone Resistance among Clinical Isolates of Methicillin-Resistant Staphylococcus aureus in Ontario, Canada, Antimicrob. Agents Chemother.,1991,35(9),1911-13

[16] Kuehnert M.J., Hill H.A., Kupronis B.A., Tokars J.I., Solomon S.L., Jernigan D.B., Methicillin-resistant Staphylococcus aureus–related hospitalizations, United States. Emerg.Infect. Dis.,2005,11:868–72

[17] Hiramatsu K., Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance, Lancet Infect. Dis.,2001,1(3),147-55

[18] Hiramatsu, K., Reduced susceptibility of Staphylococcus aureus to vancomycin — Japan 1996, Morb. Mortal. Wkly. Rep.,1997,27,624–26

[19] Centers for Disease Control and Prevention S. aureus resistant to Vancomycin in the US, Morb. Mortal. Wkly. Rep., 2002, 51, 565-67

[20] Centers for Disease Control and Prevention, Vancomycin resistant S. aureus Pennysylvania 2002, Morb. Mortal. Wkly. Rep.,2002, 51, 902

[21] Riley T.V., Pearman J.W., Rouse I.L., Changing epidemiology of methicillin-resistant Staphylococcus aureus in Western Australia, Med. J. Aust.,1995, 163,412–4

[22] Cookson B.D., Methicillin-resistant Staphylococcus aureus in the community: new battlefronts, or are the battles lost?, Infect. Control Hosp. Epidemiol., 2000, 21, 398–403

[23] Chambers H.F., Community associated Methicillin resistant Staphylococcus aureus- resistance and virulence coverage. N. Engl. J. Med., 2005, 325, 1485-87

[24] Moran G.J., Krishnadasan A., Gorwitz R.J. Fosheim G.E., McDougal L.K., Carey R.B. et al., Methicillin resistant S. aureus infections among patients in the emergency department, N. Engl. J Med., 2006, 355, 666-74

[25] Moreno F., Crisp F., Jorgenson J.H., Patterson and Patterson J.E., Methicillin-resistant Staphylococcus aureus as a community organism,Clin. Infect. Dis., 1995, 21, 1308-12.

[26] Bukharie H.A., Abdelhadi M.S., Saeed I.A., Rubaish A.M., Larbi E.B., Emergence of methicillin- resistant Staphylococcus aureus as a community pathogen. Diagn.Microbiol.Infect.Dis., 2001, 40,1-4

[27] Salgado C.D., Farr B.M., Calfee D.P., Community acquired methicillin-resistant Staphylococcus aureus: A meta-analysis of prevalence and risk factors, Clin.Infect. Dis., 2003, 36, 131-9

[28] Adcock P.M., Pator P., Medly F., Patterson J.E., Murphy T.V., Methicillin- resistant Staphylococcus aureus in two child care centers, J. Infect. Dis., 1998, 178, 577-80

[29] Lindenmayer J.M., Schoenfeld S.,O'Grady R.,CarneyJ.K., Methicillin-resistant Staphylococcus aureus in a high school wrestling team and the surrounding community, Arch.Intern. Med.,1998,158, 895-9

[30] Centers for Disease Control and Prevention. Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections—Los Angeles County, California, 2002-2003, Morb. Mortal. Wkly. Rep., 2003, 52, 88

[31] Cohen P.R., Kurzrock R., Community-acquired methicillin-resistant Staphylococcus aureus skin infection: an emerging clinical problem, J. Am. Acad. Dermatol., 2004, 50,277-80

[32] Olayinka B.O., Olnitola O.S., Olayinka A.T., Raji B., Antibiotic susceptibility pattern and multiple antibiotic resistance wider of S. aureus isolates in Zaria, Nigeria, J. Trop. Biosci., 2004, 451-4

[33] Kowalski T.J., Berbari E.F., Osmon D.R., Epidemiology, treatment and prevention of community acquired methicillin resistant Staphylococcus aureus infections, Mayo Clin. Proc., 2005, 80(9), 1201-08

[34] Francis J.S., Doherty M.C., Lopatin U., Johnston C.P.,Sinha G., Ross T., Severe community onset pneumonia in healthy adults caused by methicillin resistant Staphylococcus aureus carrying panton valentine leukocidin genes, Clin. Infect. Dis.,2005, 40(1),100-7

[35] O'Brien F.G.,Pearman J.W.,Gracey M.,Riley T.V., Grubb W.B., Community strains of MRSA involved in hospital outbreak, J. Clin. Microbiol. 1999, 37(9), 2858-62

[36] Pelag A.Y. and Munckhof W.J., Fatal necrotizing pneumonias due to community acquired Methicillin resistant Staphylococcus aureus, Med. J. Aus., 2004,181(4), 228-29

[37] Klevens M.R., Morrison A., Nadle J., Petit S., Gershman K., Ray S. et al., Invasive Methicillin resistant Staphylococcus aureus infections in the United States, JAMA, 2007, 298,1763-71

[38] Fuda C.,Suvorov M., Vakulenko S.B., Mobashrey S., The Basis for Resistance to β-Lactam antibiotics by Penicillin-binding Protein2a of Methicillin-resistant Staphylococcus aureus, J. Biol. Chem., 2004, 279(39): 40802-6

[39] Brown D.F.J., Reynolds P.E., Intrinsic resistance to beta-lactam antibiotics in Staphylococcus aureus. FEBS Lett.,1980,122,275-78

[40] Hartman B.J., Tomasz, A., Low-affinity penicillin binding protein associated with β-lactam resistance in Staphylococcus aureus. J. Bacteriol.,1984,58, 513-16

[41] Georgopapadakou N.H., Dix B.A., Mauriz, Y.R., Possible physiological functions of penicillin-binding proteins in Staphylococcus aureus, Antimicrob. Agents Chemother., 1986; 29, 333-36

[42] Utsui Y., Yokota T., Role of altered penicillin binding protein in methicillin and cepham resistant Staphylococcus aureus. Antimicrob. Agents Chemother., 1985, 28, 397-403

[43] Song M.D., Wachi M., Doi M., Ishino F., Matsuhashi M., .Evolution of inducible penicillin resistant target protein in methicillin resistant S. aureus by gene fusion, FEBS Lett.,1987,221, 167-71

[44] Walsh T.R., Howe R.A., The prevalence and mechanisms of vancomycin resistance in Staphylococcus aureus, Ann. Rev. Microbiol., 2002, 56, 657–75

[45] Chopra I., Antibiotic resistance in Staphylococcus aureus: Causes, concerns and cures?, Exp. Rev. Ant. Infect. Ther., 2003, 1(1), 45-55

[46] Weisblum B., Erythromycin resistance by ribosome modification, Antimicrob. Agents Chemother., 1995,39,577-85

[47] Ross J.I., Eady E.A., Cove J.H., Canliffe W.J., Cunliffe W.J., Baumberg S., Wootton J.C., Inducible erythromycin resistance in Staphylococci is encoded by a member of ATP binding transport supergene family, Mol. Microbiol.,1990, 4, 1207-14

[48] Saxena, S., Combating multidrug resistance microbes: A burgeoning problem In Microbes & Human Health, Vol.4, 2007; Edited by Dr. A.K.Chauhan, Dr.Harsha Kharkwal & Dr. Ajit Varma,pp.589-607,I.K.International Publishing House, New Delhi,India ISBN81-89866-05-02

[49] Bismuth R., Zilhao R., Sakamoto H. Guesdon J.L., Courvalin P., Gene heterogeneity for tetracycline resistance in Staphylococcus spp. Antimicrob. Agents Chemother., 1990,34,1611-14

[50] Warsa U.C., Nonoyama M., Ida T., Okamoto R., Okubo T., and Shimauchi C., et.al., Detection of tet (K) and tet(M) in Staphylococcus aureus of Asian countries by Polymerase chain reaction, J. Antibiot., 1996, 49(11),1127-32

[51] Trzcinski K., Cooper B.S., Hryniewicz W., Dawson C.G., Expression of resistance to tetracyclines in strains of methicillin- resistant Staphylococcus

aureus, J. Antimicrob. Chemother., 2000, 45, 763-70
[52] Schimtz, F.J., Angela K., Sadurski, R. , Milatovic D, Fluit A.C., et al. Resistance to tetracycline and distribution of tetracycline resistance genes in European Staphylococcus aureus isolates. J. Antimicrob. Chemother., 2001, 47, 239-40
[53] Shinabarger D.L., Marotti K.R., Murray R.W., Lin A.H., Melchior E.P., Swaney S.M. et al. Mechanisms of actions of oxazlidinones: effects of linezolid and eperezolid on translation reactions. Antimicrob. Agents Chemother., 1997, 41, 2132–6
[54] Tsiodras S., Gold H.S., Sakouloulas G., Eliopoulos P.M.,Wennersten C., Venkataraman L., et al., Linezolid resistance in a clinical isolate of Staphylococcus aureus, Lancet, 2001, 358, 207-8
[55] Kloss P., Xiong L., Shinabarger D.L., Mankin, A.S. Resistance mutations in 23 S rRNA identify the site of action of the protein synthesis inhibitor linezolid in the ribosomal peptidyl transferase center, J. Mol. Biol.,1999, 294(1), 93-101
[56] Vanuffel P., Giambattista Di M., Cocito, C., Chemical probing of virginiamycin M –promoted conformational change of the peptidyltransferase domain, Nuc. Acids Res.,1994, 22, 4449-53
[57] Mitchell B.A., Brown M.H., Skurray, R.A., Qac A efflux pumps from Staphylococcus aureus: Comparative analysis of resistance to diamidines, biguanidines and guanylhydrazones, Antimicrob. Agents Chemother., 1998, 42, 475-71
[58] Walmsley M.B., Mckeegan K.S., Walmsley, A.R., Structure and function of efflux pumps that confer resistance to drugs, Biochem. J.,2003, 376,313–38
[59] Allignet J., Solh N.El., Characterization of a new staphylococcal gene, vga B, encoding a putative ABC transfer conferring resistance to streptogramin A and related compounds,Gene,1997, 202, 133-38
[60] Prunier A.L., Malbruny B., Laurans M., Brouard J., Duhamel J.F. and Leclercq R., High rate of macrolide resistance in Staphylococcus aureus strains from patients with cystic fibrosis reveals high proportions of hypermutable strains, J. Infect. Dis., 2003, 187, 1709–16
[61] Trucksis M., Wolfson J.S., Hooper D.C., A novel locus conferring fluoroquinolone resistance in Staphylococcus aureus, J Bact., 1991, 173(18), 5854-60
[62] Ng, E.Y., Trucksis, M., Hooper, D.C., Quinolone resistance mutations in topoisomerase IV: relationship to the flqA locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in Staphylococcus aureus, Antimicrob. Agents Chemother.,1996, 40, 1881-88
[63] Allignet J., Loncle V., Simenel C., Delepierre M., El Solh N., Sequence of a staphylococcal gene, vat, encoding an acetyltransferase inactivating the A-type compounds of virginiamycin-like antibiotics, Gene,1993,130, 91-98
[64] McGowan, J.E. Jr, Gerding D.N., Does antibiotic restriction prevent resistance?, New Horiz.,1996,4: 370-6
[65] Niederman M.S., Is “Crop rotation” of antibiotic the solution to a “resistant” problem to ICU?, Am. J. Resp. Crit. Care Med., 1997; 156: 1029-31
[66] Jarvis, W.R., Handwashing- the Semmelweis lesson forgotten?, Lancet , 1994, 344 (8933): 1311-2
[67] Casewell, M.W., Hill R.L., Minimal dose requirements for nasal mupirocin and its role in the control of epidemic MRSA., J. Hosp. Infect., 1991;19: 35-40
[68] Jones M.E., In vitro profile of a new β-lactam, Ceftobiprole with activity against MRSA., Clin. Microbiol. Infect., 2007, 13(2):17-24
[69] Koga T., Abe T., Harumi I., Takenouchi T., Kitayama A., Yoshida T., et al. In vitro and in vivo antibacterial activities of CS-023 (RO4908463), a novel parenteral carbapenem. Antimicrob. Agents Chemother., 2005, 49(8), 3239-3250
[70] Sum P.E., Lee V.J., Testa R.T., Hlavka J.J., Ellestad G.A., et al. , Glycylcyclines - A new generation of potent antibacterial agents through modification of 9-aminotetracyclines, J. Med. Chem., 1994, 37, 184-88
[71] Goldstein F.W., Kitzis M.D., Acar,J.F.N., N-Dimethylglycylamido derivatives of minocycline and 6-demethyl-6-desoxytetracycline. Two new glycylcyclines highly effective against tetracycline-resistant gram-positive cocci., Antimicrob. Agents Chemother., 1994, 38, 2218-20
[72] Livermore D.M., Tigecycline: what is it, and where should it be used?,J.Antimicrob. Chemother., 2005,56(4), 611-14
[73] Bergeron J., Ammirati M., Danley D., Glycylcyclines bind to the high-affinity tetracycline ribosomal binding site and evade Tet (M),-and Tet(O)-mediated ribosomal protection, Antimicrob. Agents Chemother., 1996, 40, 2226-8
[74] Harris, R. and Cruz, M., Tigecycline (Tygacil): A Novel First-in-Class, Broad-Spectrum Intravenous Antibiotic For the Treatment of Serious Bacterial Infections, Pharmacy & Therapeutics, 31(1), 18-27and 57

[75] McKenney D., Quinn J.M., Jackson C.L., Guilmet J.L., Landry J.A., Tanaka S.K., et al. Evaluation of PTK 0796 in experimental model of infections caused by gram positive and gram-negative pathogen. Abstr. InterSc. Conf. Antimicrob. Agents Chemother., 2003, Sep 14-17 , abstract no. F-757

[76] Shah, P.M., The need for new therapeutic agents: what is in the pipeline?,Clinical Microbiol.Infect., 11, 36–42

[77] Zeckel M.L., Preston D.A., Allen B.S., In vitro activities of AntiLY333328 and comparative agents against nosocomial gram positive pathogens collected in a 1997 global surveillance study, Antimicrob. Agents Chemother., 2000, 44(5), 1370-68

[78] Barrett J.F., Oritavanacin: Eli Lilly & Co., Curr. Opinion Investig. Drugs, 2001, 2(8), 1039-44

[79] Jabés D., Candiani G., Romanó G., Brunati C., Riva S., and Cavaleri,M. , Efficacy of Dalbavancin against Methicillin-Resistant Staphylococcus aureus in the Rat Granuloma Pouch Infection Model. Antimicrob. Agents Chemother., 48(4), 1118-23

[80] Lin G., Credito K., Ednie L.M. and Appelbaum, P.C., Antistaphylococcal activity of Dalbvancin, an experimental glycopeptide, Antimicrob. Agents Chemother., 2005,49(2), 770-72

[81] O'Hare M.D., Ghosh G., Felmingham D. and Grüeberg R.N., In vitro studies with ramoplanin (MDL 62,198): a novel lipoglycopeptide antimicrobial, J. Antimicrob.Chemother.,1990,25,217-20

[82] Montecalvo M.A., Ramoplanin: a novel antimicrobial agent with the potential to prevent vancomycin-resistant enterococcal infection in high-risk patients, J. Antimicrob.Chemother., 2003, 51(Suppl. S3), iii31–iii35

[83] Takahata M., Mitsuyama J., Yamashiro Y., Yonezawa M., Araki H., Todo Y., et al., In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone, Antimicrob. Agents Chemother., 1999, 43:1077-84

[84] Noviello S., Ianniello F., Leone S. and Esposito S., Comparative activity of garenoxacin and other agents by susceptibility and time–kill testing against Staphylococcus aureus, Streptococcus pyogenes and respiratory pathogens, J. Antimicrob. Chemother., 2003, 52,869-72

[85] Schmitz FJ, Fluit AC, Milatovic D, Verhoef J., Heinz H.P. and Brisse S., In vitro potency of moxifloxacin, clinafloxacin and sitafloxacin against 248 genetically defined clinical isolates of S.aureus, J. Antimicrob. Chemother., 2006, 46, 109-13

[86] Bhagwat SS, Mundkar LA, Gupte SV, Patel M.V., and Khorakiwala H.F., The anti- MRSA quinolone WCK771 has potent activity against sequentially labeled mutants and has a narrow mutant selection windows against quinolone resistant S. aureus and preferentially targets DNA gyrase. Antimicrob. Agents Chemother., 2006, 50(11), 3568-79

[87] Das B., Rudra S., Yadav A., Ray A., Raja Rao A.V.S., Srinivas A.S.S.V., et al., Synthesis and SAR of novel oxazolidinones: discovery of ranbezolid, Bioorg.Med.Chem.Lett., 2005, 15(19), 4261-7

[88] Mathur T., Bhateja P., Pandya M., Fatma T., Rattan A., In vitro activity of RBx 7644(ranbezolid) on biofilm producing bacteria, Int.J. Antimicrob. Agents, 2004, 24(4),369-73

[89] Rattan A., RBx-7644: Oxazolidinone antibacterial, Drugs of the future, 2003, 28(11),1070-77

[90] Bush K., Macielag M. and Weidner-Wells, M., Taking inventory: antibacterial agents currently at or beyond Phase 1, Curr.Opin. Microbiol., 2004, 7(5), 466-76

[91] Gill C.J., Abruzzo G.K., Flattery A.M., Misura A.S., Bartizal K., Hickey E.J., In Vivo Efficacy of a Novel Oxazolidinone Compound in Two Mouse Models of Infection, Antimicrob. Agents Chemother., 2007, 51(9), 3434-36

[92] Yuan Z.,Trias J.,White R.J., Deformylase as a novel antibacterial target, Drug Discovery Today, 2001,6(18), 954-61

[93] Credito K, Lin G, Ednie LM and Appelbaum P.C., Antistaphylococcal activity of LBM415, a new peptide deformylase inhibitor, compared with those of other agents, Antimicrob. Agents Chemother. 2004, 48, 4033–6

[94] Hoang T.T., Schweizer H.P., Fatty acid biosynthesis in Pseudomonas aeruginosa: cloning and characterization of the fabAB operon encoding ß-hydroxydecanoyl-acyl carrier protein dehydratase (FabA) and ß-ketoacyl-acyl carrier protein synthase I (FabB). J Bacteriol., 1997,179, 5326-32

[95] Heath R.J., Rubin J.R., Holland D.R., Zhang E., Snow M.E., and Rock C.O., Mechanism of Triclosan Inhibition of Bacterial Fatty Acid Synthesis, J. Biol. Chem. 1999, 274(16), 11110-14

[96] Payne D.J., Warren P.V., Holmes D.J., Ji Y., Lonsdale J.T., Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery, Drug Discovery Today, 2001, 6(10), 537-44

[97] Oh K.B., Oh M.N., Kim J.G., Shin D.S., Shin J, Inhibition of sortase-mediated Staphylococcus aureus adhesion to fibronectin via fibronectin-binding protein by sortase inhibitors, Appl. Microbiol. Biotechnol., 2005, 70(1),102-6

[98] Kim S.H., Shin D.S., Oh M.N., Chung S.C., Lee

J.S., Chang I.M., Oh K.B., Inhibition of Sortase, a Bacterial Surface Protein Anchoring Transpeptidase, by β-Sitosterol-3-O-glucopyranoside from Fritillaria verticillata, BioSci. Biotech. Biochem., 2003, 67(11): 2477-79

[99] Riedlinger J., Reicke A., Zähner H., Krismer B., Bull A.T., Maldonado L.A., Ward A.C., Goodfellow M., Bister B., Bischoff D.,Süssmuth R.D.,Fiedler H.P. , "Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine Verrucosispora strain AB-18-032" J. Antibiot., 2004, 57, 271-79

[100] Allsop A.E., New Antibiotic discovery, novel screens, novel targets and impact of genomics, Curr. Opin. Microbiol.,1998, 1(5),530-34

[101] Dunman PM, Murphy E, Haney S, Kellogg G.T.,Wu S., et al., Transcription Profiling-Based Identification of Staphylococcus aureus Genes Regulated by the agr and/or sarA Loci, J. Bacteriol. 2001, 183, 7341-53

[102] Liu J., Dehbi M, Moeck G, Arhin F., Bauda P., Bergeron D., et al. , Antimicrobial drug discovery through bacteriophage genomics, Nature Biotech., 2004, 22(2), 185-91

[103] Moreillon P.,The efficacy of amoxicillin /clavulanate (Augmentin) in treatment of severe staphylococcal infections, J Chemother.1994, 6(2). 51-7

[104] Prieto J, Aguilar L, Gimenez MJ, Toro D., Gómez-Lus M. L., Dal-Ré R., et al. In vitro Activities of co-amoxiclav at concentrations achieved in human serum against the resistant subpopulation of heteroresistant Staphylococcus aureus: a Controlled Study with vancomycin, Antimicrob. Agents Chemother.,1998,42 (7),1574-1577

[105] Edouard R.S., Pestel-Caron M., Lemeland J.F., Caron F., In vitro synergistic effects of double and triple combinations of β-Lactams, vancomycin, and netilmicin against methicillin-resistant Staphylococcus aureus strains, Antimicrob. Agents Chemother., 2004, 44(11), 3055-60

[106] Shelburne S.A., Musher D.M., Hulten K., Ceasar H., Lu M.Y., Bhaila I., et al. In vitro killing of community-associated methicillin-resistant Staphylococcus aureus with drug combinations, Antimicrob. Agents Chemother., 2004, 48, 4016–9

[107] Rand K.H. and Houck H., Synergy of daptomycin with oxacillin and other β-Lactams against methicillin resistant Staphylococcus aureus, Antimicrob. Agents Chemother., 2004,48(8),2871-75

[108] Kono K., Tatara I., Takeda S., Arakawa K., Shirotani T., Okada M. et al. Antibacterial activity of epigallocatechin gallate methicillin resistant Staphylococcus aureus. Journal of Japan Association of Infectious Diseases,1994,68,1518-22

[109] Shiota S., Shimizu M., Mizushima T., Ito H., Hatano T., Yoshida T., Tsuchiya T., Marked reduction in the minimum inhibitory concentration (MIC) of beta-lactams in methicillin-resistant Staphylococcus aureus produced by epicatechin gallate, an ingredient of green tea (Camellia sinensis)., Biol. Pharm. Bull.,1999, 22(12), 1388-90

[110] Takahashi O., Cai Z., Toda M., Hara Y., Shimamura T. et al., Appearance of antibacterial activity of oxacillin againt methicillin resistant Staphylococcus aureus (MRSA) in the presence of catechin. Journal of Japan Association of Infectious Diseases, 1995,69: 1126-34

[111] Hamilton- Miller J.M, Shah S., Activity of tea component epicatechin gallate and analogues against methicillin resistant Staphylococcus aureus, J Antimicrob. Chemother., 2000, 46, 852-53

[112] Zhao W.H., Hu Z., Okubo S., Hara Y. and Shimamura T., Mechanism of synergy between epicatechin gallate and β-Lactams against methicillin resistant Staphylococcus aureus, Antimicrob. Agents Chemother., 2001, 45(6), 1737-42

[113] Nicolson K., Evan G. and O'Toole P.W., Potentiation of methicillin activity against methicillin-resistant Staphylococcus aureus by diterpenes, FEMS Microbiol.Lett.,1999, 179(2), 233–39

[114] Smith E.C.J., Kaatz G.W., Seo S.M., Wareham N.,Williamson E.M. et al., The phenolic diterpene totarol inhibits multidrug efflux pump activity in S. aureus. Antimicrob. Agents Chemother.,2007, 51(12): 4480-83

[115] Schmitz F.J., Fluit A.C., Luckefahr M., Engler B., Hofmann B., Verhoef J. et al. The effect of reserpine, an inhibitor of multidrug efflux pumps, on the in vitro activities of ciprofloxacin, sparfloxacin and moxifloxacin against clinical isolates of Staphylococcus aureus, J. Antimicrob. Chemother.,1998, 42, 807-810

[116] Gibbons S. and Udo E.E., The effect of reserpine, a modulator of multidrug efflux pumps on the in vitro activity of tetracycline against clinical isolates of methicillin resistant Staphylococcus aureus possessing tet(k) determinant, Phytother. Res., 2000, 74,139-140

[117] Stermitz F.R., Lorenz P., Tawara J.N., Zenewicz L.A., Lewis K., Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5-methoxyhydnocarpin, a multidrug pump inhibitor, Proc. Natl. Acad. Sci. USA, 2000,97,1433–37

[118] Stermitz F.R., Matsuda J.T., Lorenz P., MuellerP., ZenewiczL., Lewis K., et al.,5-Methoxy-hydnocarpin and pheophorbide A: Berberis species components

which potentiate berberine growth inhibition of resistant Staphylococcus aureus. J. Nat. Prod., 2000, 63, 1146–49

[119] Oluwatuyi M., Kaatz G.W. and Gibbons S., Antibacterial and resistance modifying activity of Rosmarinus officinalis, Phytochem., 2004, 65(2) 3249-54

[120] Fujita M., Shiota S., Kuroda T., Tsutomu H., Takashi Y., Tohru M., et al., Remarkable synergies between baicalein and tetracycline and baicalein and β-Lactams against methicillin resistant Staphylococcus aureus, Microbiol. Immunol., 2005, 49, 391-396

[121] Khan I.A., Mirza Z.M., Kumar A., Verma V., Qazi G.N., Piperine, a phytochemical potentiator of ciprofloxacin against Staphylococcus aureus. Antimicrob. Agents Chemother., 2006, 50(2), 810-12

[122] Smith P., Stewart J., Fyfe L., Influence of subinhibitory concentrations of plant essential oils on the production of enterotoxins A and B and α-toxin by Staphylococcus aureus, J. Med. Microbiol., 2004, 53, 1023–27

[123] Dickson R.A., Houghton P.J., Hylands P.J., Gibbons S., ; Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of Mezoneuron benthamianum Baill., Securinega virosa Roxb. & Wlld. and Microglossa pyrifolia Lam., Phytother. Res., 2006, 20, 41-45

[124] Braga C., Leite A.A.M., Xavier K.G.S., Takahashi, J A., Bemquerer, M P., Chartone-Souza E., et al. Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus, Can. J Microbiol., 2005, 51(7), 541–47

[125] Okusa P.N., Penge O., Devleeschouwer M., Duez P., Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wild (Boraginaceae), J. Ethnopharmacol., 2007, 112(3), 476-81

[126] Wright G.D., Resisting resistance: New chemical strategies for battling superbugs,Chem. Biol., 2000,7, R127-32

[127] Kristiansen J.E., The antimicrobial activity of psychotherapeutic drugs and stereo-isomeric analogues, Dan. Med. Bull.,1990,37,165-82

[128] Kristiansen J.E., Chlorpromazine: non-antibiotics with antimicrobial activity—new insights in managing resistance?, Curr.Opin.Investig. Drugs,1993, 2, 587-91

[129] Kristiansen J.E., Amaral L., The potential management of resistant infections with non-antibiotics, J. Antimicrob. Chemother., 1997, 40, 319-27

[130] Kaatz G.W., Moudgal V.V., Seo S.M, Kristiansen J.E., Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in Staphylococcus aureus, Antimicrob. Agents Chemother., 2003, 47, 719-26

[131] LAM K.S.,Discovery of novel metabolites from marine actinomycetes, Curr. Opin. Microbiol. 2006, 9, 245-51

[132] Gomber C., Saxena S.. Anti-staphylococcal potential of Callistemon rigidus, Central European Journal of Medicine, 2007, 2(1),79-88

[133] Katarere D.R., Eloff J.N., Antibacterial and Antioxidant activity of Sutherlandia frutescens (Fabaceae) a reputed anti HIV/AIDS phytomedicine, Phytother. Res., 2005, 19(9), 779-81

[134] Akinyemi K.O., Oladapo O., Okwara C.E., Ibe C.C. and Fasure K.A., Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity, BMC Complementary and Alternative Medicine,2005, 5, 6 (doi:10.1186/1472-6882-5-6)

[135] Nitta T., Arai T., Takamatsu H., Inatomi Y., Murata H., Iinuma M., Tanaka T., ItoT., Asai F., Ibrahim I., Nakanishi T. and Watabe K., Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin-Resistant Staphylococcus aureus, Jour. Health Sciences,. 2002, 4, 273-76

[136] Gibbons S., Anti-Staphylococcal plant natural products, Nat. Prod. Rep., 2004, 21, 263–77

[137] Schempp C.M., Pelz K., Wittmer A., Schöpf E., Simon J.C., Antibacterial activity of hyperforin from St John's wort, against multiresistant Staphylococcus aureus and gram-positive bacteria, Lancet, 1999, 353(9170), 2129

[138] Iinuma M., Tosa H., Tanaka T., Asai F., Kobayashi Y., Shimano R., Miyauchi K., Antibacterial activity of Xanthones from guttiferous plants against Methicillin resistant Staphylococcus aureus, J. Pharm. Pharmacol.,1996,48(8), 861-65

[139] Keller M. and Zengler K. Tapping into microbial diversity, Nature Rev. Microb. 2004, 2(2),141–150.

[140] Demain A.L., Microbial natural products: Alive and well in 1998, Nature Biotechnol., 1998, 16, 3-4

[141] Demain A.L., Pharmacologically active secondary metabolites of microorganisms, Appl. Microbiol. Biotechnol., 1999, 52, 455–63

[142] Tulp M. and Bohlin L. Functional versus chemical diversity: is biodiversity important for drug discovery?, Trends Pharmacol. Sci., 2002, 23, 225–31

[143] Wang J., Soisson S.M.,Young K., Shoop W., Kodali S., Galgoci A., Painter R.,et al., Platensimycin is

a selective Fab F inhibitor with potent antibiotic properties, Nature, 2006, 441: 358–61

[144] Bull A.T., Stach J.E., Ward A.C., Goodfellow M., Marine actinobacteria: Perspectives, challenges, future directions, Antonie Van Leeuwenhoek, 2005, 87, 65–79

[145] Strobel G. and Daisy B., Bioprospecting for microbial endophytes and their natural products, Microb. Mol.Biol. Rev. 2003, 67(4), 491-502

[146] Castillo U., Strobel G.A., Ford E.J., Hess W.M., Porter H., Jensen J.B., et.al., Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on Kennedia nigricans, Microbiol., 2002, 148, 2675-85

[147] Lee L.Y, Miyamoto Y.J., McIntyre B.W., Hook M., McCrea K.W., McDevitt D., Brown E.L., The Staphylococcus Map Protein is an immunomodulator that interferes with T-cell mediated responses, J. Clin. Invest., 2002, 110, 1461–71

[148] Shinefield H., Black S., Fattom A., Horwith G., Rasgon S., Ordonez J.,et al., Use of a Staphylococcus aureus conjugate vaccine in patients receiving hemodialysis, N. Engl. J. Med., 2002, 346, 491-6

[149] Burnie J.P., Matthews R.C., Carter T., Beaulieu E., Donohoe M., Chapman C., Williamson P. and Hodgetts S.J., Identification of an Immunodominant ABC Transporter in Methicillin-Resistant Staphylococcus aureus Infections, Infect. Immun., 2000, 68(6), 3200–9

[150] Schuhardt V.T., Schindler C.A., Lysostaphin therapy in mice infected with Staphylococcus aureus., J Bacteriol., 1964,88, 815–16

[151] Kusuma C.M., Kokai-Kun J.F., Comparison of Four Methods for Determining Lysostaphin Susceptibility of Various Strains of Staphylococcus aureus, Antimicrob. Agents Chemother., 2005, 49, 3256-63

[152] Kokai-Kun J.F., Walsh S.M., Chanturiya T., Mond J.J., Lysostaphin cream eradicates Staphylococcus aureus nasal colonization in a cotton rat model. Antimicrob. Agents Chemother., 2003, 47(5),1589–97

[153] Yang X., Cong-Ran L., Ren-Hui L., Wang Y.M., Zhang W.X., ChenH.Z., et al., In vitro activity of recombinant lysostaphin against Staphylococcus aureus isolates from hospitals in Beijing, China Journal of Medical Microbiology, 2007, 56, 71–76

[154] Kiri N., Gordon A., Climo M.W., Combinations of Lysostaphin with β-Lactams are synergistic against oxacillin-resistant Staphylococcus epidermidis,Antimicrob.Agents Chemother., 2002, 46 (6), 2017–20

[155] Vavra S.B., Roberta B.C., Robert S.D., Development of vancomycin and lysostaphin resistance in a methicillin-resistant Staphylococcus aureus isolate, J. Antimicrob. Chemother., 2001,48, 617–25

[156] Stranden A., Ehlert K., Labischinski H., Berger-Bachi B., Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in a fem AB null mutant of methicillin-resistant Staphylococcus aureus,J. Bacteriol.,1997,179, 9-16

[157] Ling B. and Berger-Bachi, B., Increased overall antibiotic susceptibility in Staphylococcus aureus femAB null mutants. Antimicrob. Agents Chemother., 1998, 42,936-38

[158] Schneider M.M.S., Berger-Bächi B., Tossi A., Sahl A.G., Wiedemann I., In vitro assembly of a complete, pentaglycine interpeptide bridge containing cell wall precursor (lipid II-Gly5) of Staphylococcus aureus, Mol. Microbiol., 2004, 53(2), 675–85

[159] Hutchinson C.R., Combinatorial biosynthesis for new drug discovery, Curr.Opin Microb.,1998, 1, 319-29

[160] Borchardt J.K., Genetic engineering may keep one of the richest drug gold mines from being played out, Modern Drug Discovery,1999, 2(4), 22-29

[161] Jacobsen J.R., Khosla C.,New directions in metabolic engineering.,Curr.Opin. Chem. Biol.,1998, 2,133-37

[162] Saxena, S. and Kumar D., Human pathogenic bacteria-plant interaction: Potential as novel antimicrobials, International Journal of Biomedical and Pharmaceutical Sciences, 2007,1(2), 120-23

[163] Baltz R.H., Vivian M. Stephen K.W., Natural products to drugs: daptomycin and related lipopeptide antibiotics, Nat. Prod. Rep., 2005, 22, 717 – 7

[164] Yin X, Zabriskie TM., The enduracidin biosynthetic gene cluster from Streptomyces fungicidicus, Microbiol., 2006, 152, 2969-83