



COMBATING MRSA-NOVEL APPROACHES & THERAPEUTIC ADVANCES

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Article Received on 07/08/2016

Article Revised on 28/08/2016

Article Accepted on 19/09/2016

ABSTRACT

Bacterial resistance is a growing concern and yet few new antibiotics active against multi-resistant bacteria are being explored, called as innovation gap. Methicillin-resistant *S. aureus* (MRSA) remains the leading cause of bacterial infections worldwide in both community and hospital setting, with a significant impact on the mortality and morbidity rates and the financial burden that is associated. This review provides an update on novel anti-MRSA molecules currently under pre-clinical and clinical development with emphasis on the new classes and the new molecules from already existing classes of antibiotics and the novel drugs as alternatives to traditional antibiotics compounds that might help us tide over this crisis. Anti-virulence approaches, phage therapy and therapeutic antibodies are strategies that may yield drugs with high specificity and narrow spectra. However, robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.

KEYWORDS: MRSA, antivirulence, antimicrobial peptide, antibiotics.

INTRODUCTION

Staphylococcus aureus (*S.aureus*) remains a leading cause of bacterial infections worldwide, ranging from minor skin and soft tissue infections to more severe conditions such as bacteremia and infective endocarditis. With the introduction of penicillin in 1940s, *S. aureus* infections were routinely and successfully treated. However, within 2 years naturally occurring penicillin-resistant strains were reported.^[1] Similarly, within two years of the introduction of methicillin, strains of methicillin-resistant *S. aureus* (MRSA) were observed.^[2] In the last century, high prevalence of MRSA across the world and paucity of effective drugs prompted the increased use of vancomycin, despite its poor bioavailability and associated toxicity.^[3] This resulted in the emergence of vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S.aureus* (VRSA), making the clinical situation grimmer.^[4]

MRSA can be defined as the strains of *S. aureus* that are resistant to the isoxazolyl penicillins such as methicillin, oxacillin and flucloxacillin. MRSA offers cross resistance to all currently licensed β lactam antibiotics.^[5] Methicillin resistance arises through the production of a supplementary penicillin binding protein (PBP), known as PBP2 or PBP2a. This compensates for the functions of the essential methicillin susceptible staphylococcal PBPs and confers resistance to most β lactams.^[6]

Infections caused by MRSA have been associated with high morbidity and mortality rates. The prevalence of methicillin resistant strains varies globally from 20% in Europe,^[6] 33-55% in the United States⁶ and 30 to 80% in India.^[7] In Indian hospitals, MRSA is one of the common cause of hospital acquired infections and different hospitals have reported methicillin resistance based on antibiotic sensitivity tests.^[7] The increased prevalence of multidrug resistant (MDR) strains of *S. aureus* is due to an irrational usage of broad spectrum antibiotics in the medical and the veterinary practice, high proximity to a large number of unlicensed drug vendors, poverty leading to incomplete dosage regimen of the antibiotics, antibiotic prophylaxis, high number of immune compromised patients, the increased use of invasive procedures, devices and inadequate infection control measures.^[8]

The continuing development of currently available antibiotic arsenals to be effective against drug resistant bacteria remains important, especially for redefining infectious disease therapy (i.e. combination products with superior efficacy over either product alone), shortening length of therapy, reducing side effects profile, and reducing drug resistance profile of currently available antibacterial products. β -Lactams, macrolides, aminoglycosides, fluoroquinolones and diaminopyrimidines are important pharmacophores for the development of newer generation antibacterials to

combat drug-resistant bacteria.^[9] However, the ultimate goal of drug development for MRSA infections is to discover novel antibacterial agents which interfere with unexploited bacterial molecular target(s). Antibiotics-based treatments result in the destruction of commensal bacteria and drug resistance, so there is need of newer targets based on the molecular basis of pathogenicity of bacteria – hence disarming the bacteria.

Despite the introduction of several anti-MRSA drugs in recent years, vancomycin still remains the gold standard for anti-MRSA therapy. Considering the need for newer, more effective treatment options for multi-drug resistant (MDR) pathogens, the Infectious disease society of America (IDSA) launched the “10x20” initiative against a range of resistant pathogens including MRSA, collectively called the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *enterobacteriaceae*) pathogens, to develop 10 new effective antimicrobial drugs to treat the infections caused by these pathogens by 2020.^[10]

In this review, besides discussing the newer antibiotics, the non antibiotic based therapies like natural molecule based agents, antimicrobial peptides, antivirulence strategies, therapeutic monoclonal antibodies, phage & lysine therapy will be discussed.

A. Newer antibiotics in existing class

Cephalosporins

Ceftaroline fosamil is a new, bactericidal, parental cephalosporin with expanded Gram-positive activity, including VRSA and MRSA. Like other beta-lactam antibiotics it works by binding to penicillin-binding-proteins (PBP) on bacterial cell walls leading to irreversible inhibition of cell-wall synthesis. The anti MRSA activity is attributed to its ability to bind PBP 2a with high affinity and to inhibit the activity of PBP 2a more efficiently than other available Beta lactams.^[11] It has been the only Food and Drugs Administration (FDA) approved cephalosporin with activity against hetero-resistant vancomycin intermediate *S. aureus* (hVISA), and VRSA. Clinical trials (CANVAS 1, CANVAS 2 & FOCUS 1, FOCUS 2 studies) have demonstrated that ceftaroline was non-inferior to the standard of care for the treatment of community acquired pneumonia (CAP) and for skin and soft structure infections (SSTI) respectively.^[12,13] It has been approved by FDA in 2010 and by European Medical Agency (EMA) in 2012 for the treatment of acute bacterial skin and soft tissue infections (ABSSSIs) and CAP. The positive attributes of ceftaroline with respect to antimicrobial stewardship programs are: the low potential for resistance development and the favourable safety and tolerability profile in clinical trials.

Ceftobiprole (formerly BAL9141), a fifth generation cephalosporin is the active component of prodrug Ceftobiprole medocaril (BAL5788), is a new member of

the pyrrolidone-3-ylidenemethyl cephem series of cephalosporin. It is structurally engineered to allow binding to PBP-2a expressed by the *mecA* cassettes. It is active against MRSA, VISA, VRSA. Studies of clinical isolates and strains derived from surveillance studies reported MIC₉₀ values for MRSA as 2 to 4 µg/ml.^[14] Ceftobiprole demonstrated an in vivo activity against a large sample of community acquired-MRSA clones identified in bone infections.^[15] To date, the results of three Phase III clinical trials investigating ceftobiprole for the treatment of complicated skin and skin structure infections (cSSSIs) and hospitalized community acquired pneumonia (CAP) have been published.^[16] It has been approved in October, 2013 in Europe for the treatment of healthcare associated pneumonia (HAP), CAP & ABSSI.^[16]

Lipoglycopeptides

Dalbavancin - It is a new lipoglycopeptide that inhibits cell wall synthesis in Gram-positive bacteria through the formation of a stable complex between its heptapeptide backbone and the D-Ala-D-Ala portion of cell wall precursors. It demonstrates in vitro activity against clinically significant Gram-positive pathogens, including methicillin susceptible *S. Aureus* (MSSA), MRSA, methicillin-susceptible *S. epidermidis* (MSSE) and methicillin-resistant *S. epidermidis* (MRSE).^[17] The main PK characteristic is the long-half-life : its terminal elimination half-life of ranges from 147 to 258 hours, allowing the once-weekly dosing that maintains its serum plasma concentrations of above the MIC of common pathogens for 7 days.^[18] Its long-half life is a result of its extensive protein binding, as well as its retention within cells. The published Phase III clinical trial results show dalbavancin to be non-inferior to linezolid for treating MRSA-associated complicated skin and skin structure infection (cSSSI) with tolerable side effects.^[19] It is the first IV antibiotic approved for the treatment of ABSSSI with two dose regimen of 1000mg followed by 500 mg 1 week later. It has been approved by US FDA in May 2014.

Oritavancin It is a semi synthetic lipoglycopeptide analogue of vancomycin with at least 3 mechanisms of actions: inhibition of transglycosylation, inhibition of transpeptidation and cell membrane disruption/interaction.^[20,21] It seems promising for the treatment of serious infections caused by Gram-positive bacteria, such as MRSA, VISA, VRSA, daptomycin non susceptible *S. aureus* and VRE. It presents a rapid concentration dependent bactericidal activity. The pharmacodynamic (PD) and pharmacokinetic (PK) profiles of oritavancin are unique and suggest that it could be effective given in a single dose. The results from two previously completed Phase III (SOLO I and SOLO II) studies and one recently published Phase II trial (SIMPLIFI) indicate single 1200 mg dose of oritavancin to be safe and non-inferior to vancomycin for treating cSSSI caused by Gram-positive organisms,

including MRSA.^[22] It has been approved by US FDA in Aug 2014.

Carbapenems

Tomopenem (CS-023) is a new carbapenem with broad-spectrum activity against Gram-positive and Gram-negative pathogens for the treatment of cSSSI and HAP. It displays a good activity against ceftazidime-resistant *Pseudomonas aeruginosa*, extended-spectrum β -lactamase (ESBL) producing *Enterobacteriaceae*, penicillin-resistant *Streptococcus pneumoniae*, *H. influenzae* and MRSA, with a low rate of spontaneous resistance.^[23] It is currently in Phase II clinical trial.

Razupenem (also known as SMP-601, PTZ601, PZ-601, or SM-216601) is a new parenteral carbapenem for the treatment of complicated skin and soft-tissue infections.^[24] It has a broad-spectrum activity against Gram-positive and Gram-negative pathogens, including MRSA, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *E. faecium* (VREF), ampicillin-resistant *Haemophilus influenzae*, and ESBL-producing bacteria even if its activity is reduced by AmpC enzymes and carbapenemases.^[24,25]

Streptogramins

Linoprostin/Flopristin (NXL-103)- derived from *Streptomyces pristinaspiralis*, is a novel, orally administered mixture of semi-synthetic streptogramins, containing linopristin and flopristin in a ratio of 70:30. Flopristin blocks an early step of protein synthesis by forming a bond with a ribosome, to prevent elongation. Linopristin blocks a later step by preventing the extension of peptide chains & causing incomplete release of chains. It is effective against MRSA, MRSE and VRE. It has been shown to be 4 times more potent than quinpristin/ dalfopristin in in vitro tests.^[26] It is under Phase II clinical trial for treatment of ABSSSI.

Fluoroquinolones

Delafloxacin (RX3341) It is an investigational oral and parental fluoroquinolone active against a variety of Gram-positive bacteria, including MRSA and quinolone-resistant strains of *Staphylococcus aureus* (QRSA).^[27] It has been designated a qualified infectious disease product (QIDP) for both ABSSI & CAP by the US FDA. Two Phase III – PROCEED studies to evaluate the efficacy and safety of delafloxacin compared with vancomycin plus aztreonam for treatment of ABSSI are undergoing.

Avarofloxacin / Acorafloxacin (JNJ-Q2) is a fluorinated 4-quinolone developed for the treatment of acute bacterial SSTI and respiratory tract infections. Rates of spontaneous development of resistance to this drug in *S. pneumoniae*, MRSA, and *Escherichia coli* were indicative of a lower potential for resistance selection than other fluoroquinolones.^[28] It is available in Intravenous & oral formulations for the treatment of

CAP & ABSSI and is being under Phase III clinical trial.

Nemonoxacin (TG-873870) is a novel C-8 methoxy non fluorinated quinolone. It targets both topoisomerase II & IV. It has demonstrated a potential antibacterial activity against ciprofloxacin resistant MRSA & VRE.^[29] It has been found to be comparable to levofloxacin in terms of clinical and bacteriological response when given in the dose of 500mg and 750 mg for 7 days for the treatment of CAP in phase II study.^[30,31] It is currently in Phase III clinical trial.

ACH 702, belongs to a class called isothiazoloquinolone, that inhibits bacterial DNA replication through inhibition of DNA gyrase and topoisomerase IV. It is highly active against MRSA including biofilms but it is being pursued only as a topical agent because of extensive metabolism when given systemically.^[32] At concentrations reaching 16 times the MIC, this compound was able to reduce the activity of stationary phase biofilm associated cells of *S. aureus* by a factor of 3 log units.^[33]

Oxazolidinones

Tedizolid (TR-701) previously known as torezolid phosphate, is an inactive prodrug that, after oral or intravenous administration, is rapidly converted to the active form (torezolid). Unlike Linezolid it has been found to be bactericidal in animal models. It is 4-32 times more potent than linezolid.^[34] Approximately 80% of linezolid resistant strains were inhibited by tedizolid at a concentration of ≤ 4 μ g/ml. It does not inhibit monoamine oxidase in vivo, therefore interactions with adrenergic, dopaminergic, serotonergic drugs are not to be expected.^[35] It has been approved by US FDA in June, 2014.

Radezolid (RX-1741) offers the potential for several incremental improvements in that it is generally two-fold more active in vitro than linezolid against the staphylococci. In a double-blind phase 2 trial, patients with suspected or confirmed Gram-positive infection (a vast majority had *S. aureus* and more than 80% had MRSA infection) were given tedizolid once a day oral doses of 200, 300 or 400 mg (randomized as 1:1:1) for 5–7 days for complicated skin and skin structure infections.^[36] Clinical cure rates in excess of 95% were achieved for MRSA as well as MSSA infections in all three dosage groups. It has been shown to achieve 11 times higher levels inside macrophages and neutrophils, a property that might be useful when applied to persistent infections with intracellular organisms.^[36,37]

Dihydrofolate Reductase (DHFR) Inhibitors

Iclaprim – is a novel diaminopyrimidine like trimethoprim and has been shown to possess strong antagonistic activity against bacterial DHFR enzyme.^[38] However, despite both exhibiting similar mode of action, iclaprim has also shown to be active against trimethoprim-resistant MRSA.^[39] Its ability to overcome the F98Y DHFR-mediated trimethoprim resistance has

been attributed to increased hydrophobic interactions, which allows it to bind and inhibit the mutant enzyme.^[39,40] In randomized double blind Phase II and phase III trial studies, iclaprim was shown to be equally safe and non-inferior to vancomycin and linezolid for treating cSSSI caused by MRSA, respectively.^[41]

Ketolides

Cethromycin is a new fluoroketolide with a reported high potency against Gram positive, as well as Gram negative organisms. It has in vitro activity against penicillin and macrolide resistant Gram positive organisms, possibly due to higher affinity for the target site on the ribosomal unit.^[42] There are two comparative studies done in patients suffering from CAP, in which cethromycin 300 mg per day was compared to clarithromycin 250 mg twice daily. It demonstrated the non inferiority of cethromycin in terms of clinical cure and radiographic improvement, in low severity CAP patients.^[43] Cethromycin completed Phase III clinical trials for the treatment of CAP and was deemed to be safe according to the FDA Advisory Committee, after dosing more than 5000 patients in clinical studies for patients with mild or moderate CAP.^[44] But more trials will be needed with more severe CAP patients before approval can be granted.

Modithromycin (EDP-420) is a bridged bicyclic ketolide. It is currently in Phase II clinical development for the treatment of respiratory tract infections. It possesses an excellent pharmacokinetic profile across multiple species, with a half-life 2–3 times longer as compared to those of clarithromycin and telithromycin. The long half-life and high systemic exposure of modithromycin supports once-daily dosage.^[45]

B. Newer class of antibiotics

Aminomethylcycline (new generation tetracyclines)

Eravacycline (TP-434) is a novel oral/ parenteral fluorocycline, similar to tigecycline. It shows broad spectrum antimicrobial activity including MRSA, ESBL, VRE.^[46] Availability of an oral formulation supports its use in sequential therapy. It is evaluated under Phase III clinical trial.

Omadacycline (PTK796) an aminomethylcycline, is a semisynthetic derivative of minocycline that has in vitro potency against Gram-positive and Gram-negative bacteria. It presents activity against MSSA/MRSA, The potency of omadacycline was not impaired by lung surfactant.^[47] It has overcome tetracycline mechanism of resistance (efflux or ribosomal Protection). In a phase 2 study of over 200 adults with SSSI initially requiring IV therapy, patients were randomized to receive 100 mg every 24h IV with 200 mg oral step-down dosing or linezolid at 600 mg every 12h IV with a 600 mg every 12h oral step-down dose. It showed comparable efficacy across all the study populations.^[47]

Heterodimeric (vancomycin/ cephalosporin) antibiotic

TD-1792 is a heterodimer antibiotic composed of vancomycin covalently tethered through a chemically stable linker to a cephalosporin moiety. Each of these moieties targets distinct sites in bacterial cell wall synthesis, namely, d-Ala-d-Ala-containing peptidoglycan precursors and transpeptidase active sites in penicillin binding proteins.^[48,49] Antibacterial activity of TD-1792 is largely unaffected by coexisting resistance mechanisms, including methicillin and oxacillin resistance in staphylococci, penicillin resistance in pneumococci, fluoroquinolone resistance in streptococci, and quinupristin-dalfopristin resistance among several tested species. The phase II study explored the safety and efficacy of TD-1792 in patients with complicated skin and skin structure infections (cSSSI) caused by suspected or confirmed Gram-positive organisms.^[50]

Neoglycosides (New generation aminoglycosides)

Plazomicin (ACHN 490) is a new compound within this class that is resistant to enzymatic inhibition. It is the first of the new generation aminoglycoside, known as neoglycoside. It inhibits bacterial protein synthesis and exhibits dose-dependent bactericidal activity. It exhibits synergy with daptomycin and ceftobiprole against MRSA, VISA.^[51] Trials on healthy volunteers have shown no evidence of ototoxicity or nephrotoxicity.

Pleuromutilin

Lefamulin (BC-3781) is a novel semisynthetic systemic agent belonging to the pleuromutilin class of antibiotics. Pleuromutilins interfere with bacterial protein synthesis via a specific interaction with the 23S rRNA of the 50S bacterial ribosome subunit. In vitro, it demonstrates clinically relevant activity against the most frequently identified Gram-positive skin pathogens, including MSSA and MRSA. Furthermore, it exhibits significant in vitro activity against coagulase-negative Staphylococcus species and community respiratory pathogens.^[52,53] So far the therapeutic administration of pleuromutilin antibiotics has been limited to veterinary medicine or to the topical route in humans, due to associated toxicities. Recently the phase 2 study in the treatment of cSSTI caused by a Gram-positive pathogen has been completed by comparing lefamulin to vancomycin. The results provided the first proof of concept for the systemic use of a pleuromutilin antibiotic in the treatment of SSSIs.^[54]

Fatty acid synthesis inhibitor

Fatty acid biosynthesis in bacteria is crucial for the production of number of lipid containing components, including the cell membrane. The differences in prokaryote and eukaryote fatty acid biosynthesis provide an opportunity for the development of new antibacterial agents. Among compounds inhibiting various steps during bacterial fatty acid synthesis are triclosan and isoniazid, the two most widely used antibacterials that target fatty acid biosynthesis (FabI) in *S. aureus* and *Mycobacterium tuberculosis*, respectively.^[55] FabI

performs the last step during fatty acid elongation and has shown to be an essential enzyme in *S. aureus*.^[56]

AFN-1252 is an experimental molecule identified during a high-throughput screening of *S. aureus* FabI inhibitors and was found to be highly active against several strains of MRSA, MSSA and *S. epidermidis*.^[57,58] AFN-1252 has recently completed a Phase II clinical trial for evaluation of its safety and efficacy in the treatment of ABSSSI caused by *S. aureus* but no results have been posted/published yet.^[59]

Fab-001 and **CG400549** are two additional molecules in this category that are currently being studied for their use in staphylococcal infections. Fab-001 (MUT056399) is a narrow spectrum antibiotic currently being developed as a potent inhibitor of *S. aureus* FabI.^[60] It is currently under Phase II trials for its use in the treatment of complicated ABSSSI, CG400549 has been demonstrated to be more potent against MRSA in comparison to linezolid and vancomycin.

C. Natural product based molecule /antibiotic

Exeporfinium chloride (XF- 73) is a phase II clinical drug candidate. It is a dicationic molecule that is synthesized from 5,15-bis(4-hydroxyphenyl) porphyrin. It is active against a broad range of Gram-positive bacteria including MRSA.^[61] Interestingly, it kills *S. aureus* much faster than the other antistaphylococcal agents. It mediates antibacterial action by its membrane-perturbing depolarisation resulting in the loss of intracellular K⁺/ATP, and has been shown to be equally effective against non-dividing *S. aureus* in biofilms.^[62] Unlike daptomycin this process does not require calcium ions (a Ca-independent mechanism). The clinical development of Exeporfinium chloride is targeted at intranasal administration to reduce the risks caused by MRSA and other Gram-positive bacteria.^[63]

GSK1322322 is a novel peptide deformylase (PDF) inhibitor in the early phase of development for treatment of complicated bacterial skin and skin structure infection and hospitalized community-associated pneumonia.^[64] PDF, a highly conserved metalloproteinase, has been observed to be critical for the maturation of proteins during translation in prokaryotic cells. Failure to remove the N-formyl group prevents the action of methionine aminopeptidase, which is essential for cell growth, but PDF is not found in mammalian protein biosynthesis.^[65] Therefore, PDF represents a selective and promising target for the development of new antibacterial agents. GSK completed studies of the safety, tolerability and efficacy of GSK1322322 in subjects with acute bacterial skin and skin structure infection in a Phase II clinical trial in 2011.^[64] It is now being evaluated for phase III clinical trial.

OligoG CF-5/20 is a potential antibacterial agent against lung infections in cystic fibrosis (CF) patients. OligoG CF-5/20 is an alginate oligomer, consisting of 1,4-linked

β -D -mannuronic acid and α -L-guluronic acid residues. This non-toxic oligosaccharide may be a very useful asset to develop combination therapy with other antimicrobial agents including anti-MRSA drugs.^[66]

Artemisia princeps is a perennial herb belonging to Asteraceae family. The ethanol extract of *A. princeps* shows antibacterial activity against MRSA. It shows dose dependent bactericidal activity, also inhibit biofilm formation and inhibit virulence gene (mec A, agr A) expression.^[67]

D. Antimicrobial peptides

Antimicrobial peptides (host defense peptides) are small molecular weight polypeptides with broad spectrum of antimicrobial activity against bacteria, Viruses and fungi. Antimicrobial peptides are generally between 12 and 50 amino acids. They are usually positively charged and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. The peptide kills bacterial cells through diverse mechanisms, including disrupting membranes, interfering with metabolism, and targeting cytoplasmic components. Of the potential antimicrobial peptides, a few have developed into clinical trial drugs to date.^[68,69]

MSI-78 was the first antimicrobial peptide that was developed into a phase III clinical trial drug. It is a synthetic 22- amino-acid analogue of magainin II, a cationic peptide isolated from the skin of the African clawed frog *Xenopus laevis*. MSI-78 acetate cream (Locilex™) was designed to treat patients with diabetic foot infections and demonstrated its efficacy in lysing MDR bacteria including MRSA, vancomycin-resistant enterococcus (VRE) and other resistant pathogens.^[70] In clinical trials, the safety profile of MSI-78 was proved. Importantly, no antimicrobial resistance against MSI-78 has been reported to date.

LTX-109 is a synthetic antimicrobial peptidomimetic (SAMP) and causes selective & ultra rapid membrane disruption. It is under Phase II evaluation for topical treatment of skin infection and nasal eradication of MRSA. It has low propensity to development of resistance and active against biofilm infection.^[71]

Brilacidin (PMX-30063) is a novel pore-forming drug belonging to 'defensin-mimetics' class of antibiotics that is currently being developed for its use in the treatment of ABSSSI caused by MRSA.^[72] It has been shown to have a potent bactericidal activity against a number of Gram-negative and Gram-positive organisms. It has immunomodulatory anti biofilm property. Results from a recently presented Phase II clinical trial indicate that brilacidin is safe and non-inferior to daptomycin in the treatment of ABSSSI caused by *S. aureus*.^[72]

E. Antivirulence strategies

The emergence and increasing prevalence of bacterial strains that are resistant to available antibiotics demand the discovery of new therapeutic approaches. Targeting bacterial virulence is an alternative approach to antimicrobial therapy that offers promising opportunities to inhibit pathogenesis and its consequences without placing immediate life-or-death pressure on the target bacterium. Targeting virulence represents a new paradigm to empower the clinician to prevent and treat infectious diseases.^[73] Most traditionally used antibiotics kill bacteria by interfering with essential cellular processes. An alternative to this approach is to disarm the pathogens, making it easier for the host innate immune system to clear the infection. Virulence is defined as the ability of a pathogen to cause disease. Some of the major targets of antivirulence research are toxins, quorum-sensing, biofilm production, type III secretion and adhesion. A large number of Gram-negative bacteria release their toxins by type III secretion, making the machinery used in this process another potential target. Type III secretion involves the formation of a needle-like structure that delivers toxins and other effector molecules directly into host cells.^[74] Quorum-sensing – another potential target – can be described as the process by which bacteria “talk” to each other. Bacteria can sense each other by taking up small molecules secreted by other bacteria nearby; in this way, bacteria can act as a population instead of as individuals.^[75] Quorum-sensing enables bacteria to form biofilms, an effective approach to becoming more resistant toward both antibiotics and host immune responses. Targeting bacterial virulence is attractive since it is specific toward pathogenic bacteria and spares the commensal flora. Since bacterial viability is not directly targeted, it is also unlikely that inhibitors of virulence would show cross-resistance with existing antibiotics or even evoke novel modes of resistance.

Diflunisal (Compound IX), is an FDA-approved non steroidal anti-inflammatory drug. This represents a new use for an old drug. It inhibits the binding of agrA (accessory gene regulator), responsible for turning on release of toxins, to an oligonucleotide corresponding to the DNA sequence of promoter P3. In principle, the formation of the specific protein-DNA complex could be blocked by the binding of diflunisal either to DNA or to agr A, inhibiting agr A mediated release of α toxin. It possesses anti- MRSA activity & potentiates the activity of other antibiotics. It is shown to sensitise MRSA to subinhibitory concentration of daptomycin.^[76]

Savirin (*Staph aureus* virulence inhibitor) is a small molecule inhibitor of agr A. It disrupts the agr mediated quorum sensing in *Staph aureus* but not in the important skin commensal *Staph epidermidis*.^[77] It has shown to prevent the development of dermonecrotic ulcers following infection with agr positive bacteria in an experimental mouse model of SSI. Also repeated exposure of Savirin did not produce resistance.

F. Therapeutic antibodies

Anti-bacterial antibodies can be divided into two categories: those that bind directly to the pathogen and those that aim to neutralize toxins or other virulence factors.^[78] Antibodies that bind directly to the bacteria usually work by opsonizing the bacteria for phagocytosis. Those that bind to virulence factors disarm the bacteria, thereby giving the host a chance to clear the infection immunologically. Therapeutic antibodies have the advantage of being very specific, which means that they do not affect the commensal flora. Intended for systemic use only, they can be produced toward any pathogen. Indications for antibodies currently in clinical trials include the prevention of *S. aureus* infection in high-risk groups.

F-598 is a human IgG mono-clonal antibody. F-598 targets against MRSA and other serious infectious diseases. Monoclonal antibodies are not expected to cause bacterial resistance to the therapy. The target of F-598 is a carbohydrate on the bacterial capsule called poly-N-acetyl glucosamine (PNAG), which mediates biofilm formation.^[79] To date, several disappointing results of IgG mono-clonal antibody therapies have been reported. However, vaccine/antibody therapies are ultimate assets to combat the pathogens (e.g. MRSA) that are resistant to current antibacterial agents.

Pagibaximab is a chimeric monoclonal antibody directed against lipoteichoic acid (LTA) moiety. LTA is an important structural component of cell wall of *Staph aureus*. It is evaluated in Phase III trial for prevention of staphylococcal sepsis in infants with low birth weight.^[80]

G. Phage & Lysin therapy

Bacteriophages (phages) are bacteria-specific viruses present in nearly all environmental conditions. Phages were discovered almost a century ago. With the advent of antibiotics, the use of phages for treatment of infections fell out of favour in antibiotics. In light of the rise of antibiotic resistance, phages & their products (lysins) are rediscovered as antibacterial bioagents. Most known phages are “lytic” phages, which ultimately lyse and kill the host bacterial cells to release their progeny.^[81] Other phages are “lysogenic” and do not kill the host, being incorporated into the bacterial genome as prophages and only attack and lyse the host organism on rare events.^[81] Because phages are the natural predators of bacteria, using them as antibiotics may seem quite straightforward. Properties that make phages good drug candidates are that they are cheap to produce and very specific.^[82] Theoretically, the safety profile of phages should be excellent because of their specific action; however, little formal clinical data are available. However, the risk of resistance development is high and there may be clinical problems with neutralization of phages by the host immune response. An alternative to using whole phage particles is to use phage lysins. These are highly potent enzymes degrading the bacterial cell

wall.^[83] Resistance development toward lysins has not yet been reported.^[83]

MV-L is a highly active staphylococcal endolysin. This enzyme was able to lyse all tested strains, even MRSA and VISA strains.^[84]

LysK is an endolysin from the staphylococcal phage K, shows broad spectrum activity against staphylococcus.^[85]

SAL-1 is active against both environmentally isolated *S.aureus* and clinically isolated MRSA. Its enzymatic activity is higher than LysK activity and has reduced MIC in comparison to LysK.^[86]

TABLE I – NEWER DRUGS AGAINST MRSA

Drug class	Drug name	Mechanism of action	Status
Cephalosporin	Ceftaroline fosamil Ceftobiprole	Cell wall synthesis inhibitor	Approved by EMA, US FDA Approved in Europe
Lipoglycopeptide	Dalbavancin Oritavancin	Peptidoglycan biosynthesis and cell membrane inhibitor	Approved in 2014 Approved in 2014
Carbapenem	Tomopenem Razupenem		Phase II Phase II
Streptogramin	Linopristin/ Flopristin	Inhibits elongation step of protein synthesis	Phase II
Fluoroquinolone	Delafloxacin Avarofloxacin Nemonoxacin ACH-702	DNA synthesis inhibitor	Phase III Phase III Phase III Phase II
Oxazolidinones	Tedizolid Radezolid	Protein synthesis inhibitor (50s)	Phase III Phase II
DHFR inhibitor	Iclaprim	Folic acid synthesis inhibitor	Phase III
Ketolide	Cethromycin Modithromycin	Inhibit translocatin process during protein synthesis	Phase III Phase II
Aminomethylcycline (New generation tetracycline)	Eravacycline Omadacycline	Bacterial protein synthesis inhibitor	Phase III Phase III
Heterodimer antibiotic	TD-1792 Cephalosporine/ vancomycin	Cell membrane inhibitor	Phase III
Neoglycoside	Plazomicin	Protein synthesis inhibitor (30s)	Phase II
Pleuromutilin	Lefamulin	Inhibits Protein synthesis via a specific interaction with 23s RNA of the 50s ribosomal subunit	Phase III
Porphyrin derivative	Exeaporfinium chloride	Cell membrane inhibitor	Phase II
Peptidyl deformylase inhibitor	GSK1322322	Protein synthesis inhibitor	Phase II

CONCLUSION

Changing epidemiology of MRSA infection & design of clinical trial will continue to shape the management of serious MRSA infection. The current pipeline of investigational agent is promising. Use of antivirulence therapy can help in overcoming the resistance. Anti-virulence approaches, phage therapy and therapeutic antibodies are fields that will yield drugs with high specificity and hence narrow spectra. Rationale use of the antibiotics is necessary.

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