Multidrug resistance mechanisms *of Staphylococcus aureus,* enterococci, *Pseudomonas aeruginosa* and update on current and emerging treatments

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Among the most significant concerns in global healthcare in the 21st century are infections caused by 'superbugs', i.e., bacteria that are resistant to many commonly used antibiotics. Antibiotic resistance initially started as a problem in hospitals, with increasing numbers of nosocomial infections normally in immune-compromised and critically ill patients, but later extended into the general community. The mechanisms of drug resistance are diverse and complex, and include a number of genetic and biological factors. This work summarises the bacterial mechanisms of drug resistance, details some of the known drug-resistant bacteria and overviews some of the current treatments and emerging strategies to address this global challenge.

Keywords: multidrug resistance; MRSA; PRSA; VRSA; new drugs, novel treatments, potential treatments.

1. Summary of bacterial genetic and physiological mechanisms of resistance

Bacterial antibiotic resistance can be intrinsic, or acquired through vertical or horizontal transmission. Horizontal transmission is a major contributor to the complication of antibiotic resistance, as it allows bacteria to transfer the resistance-encoding genes even between different species by conjugation, transformation and transduction. Resistance genes are normally located on transposons that can jump from plasmid to plasmid (Sefton 2002). Recently, Ellison et al. (2018) have shown for the first time in a real time recording that bacteria also actively 'fish' for DNA from surrounding dead bacteria by scanning the environment using their pili. Vertical gene transfer is the ability of organisms to pass their genetic material to their progeny, therefore the acquired resistance gene can be permanently maintained within the species. The physiological basis of resistance includes antibiotic transformation or destruction, antibiotic active efflux, and receptor modification. Antibiotic transformation or destruction is a common strategy and may be one of the oldest mechanisms of resistance, affecting several antibiotics, especially β-lactam antibiotics, by the production of β-lactamase (Jacoby & Munoz-Price 2005). Antibiotic active efflux is another way to deal with drugs that target intracellular components and was first discovered in macrolide and tetracycline resistance (Roberts 1996, Ross et al. 1990). The pathogens express active transport modules on their plasma membrane that can pump out the drug molecules penetrating into the cell, until an ineffective concentration has been reached. Finally, receptor modification leads to poor binding of the antibiotic to the intracellular target; e.g., modifications of penicillin-binding proteins (PBPs) observed in certain types of penicillin resistance.

2. Antibiotic-resistant microorganisms

Multidrug resistance (MDR) is defined as non-susceptibility to one or more antimicrobial drugs of at least three classes (Kallen&Srinivasan 2010). Among the list of resistant bacteria, certain species are the leading cause of nosocomial infections worldwide. These are referred to as the ESKAPE pathogens, i.e., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa and Enterobacter* species. *Most of these are multidrug resistant. Among the ESKAPE pathogens, the emergence and resistance mechanisms of S. aureus, P. aeruginosa and Enterococcus will be discussed further.*

2.1 Penicillin-resistant *S. aureus* and methicillin resistant *S. aureus* (PRSA, MRSA)

S. aureus, a Gram-positive coccus, is part of our normal skin microbiota, especially the nose and perineum. Generally, its carriage rates are high and transmission is airborne or through direct contact. *S. aureus* can cause a wide range of infections, from minor skin and soft-tissue infections, chronic bone infections to life threatening bacteremia and endocarditis (Mitchell & Howden 2005). In the mid-20th century, *S. aureus* was treated effectively with penicillin and methicillin. However, due to excessive use, penicillin-resistant *S. aureus* (PRSA), then methicillin-resistant *S. aureus* (MRSA), started to emerge soon after. The time frame of development of resistance in *S. aureus* is shown in Figure 1. The first MRSA infection was noted in 1961, two years after methicillin was introduced, and the issue has become more prevalent since 1985 (Barada et al. 2007). MRSA accounts for a significant percentage of hospital-associated infections globally. In Europe, MRSA accounted for around 44% of nosocomial infections in 2008 (Kock et al. 2010). In the USA there was an estimate of 80,461 cases of invasive MRSA infection, with 11,285 deaths per year (https://www.cdc.gov/drugresistance/biggest_threats.html, last accessed July, 2018). In Thailand, Indrawattana et al.

(2013) reported that up to 60.9% of 92 *S. aureus* isolates from Prince of Songkhla Hospital and Hospital for Tropical Diseases were MRSA. MRSA produces a number of toxins such as α, β, γ, and δ enterotoxins and Panton-Valentine leukocidin which increases their virulence, and the mortality rate of MRSA bacteremia can be up to 40% (Durai, Ng Philip&Hoque 2010). Recently, the emergence of community-associated MRSA (CA-MRSA) has increased the threat, as CA-MRSA can cause infection not in only immune-suppressed but also healthy individuals (Chambers & DeLeo 2009).

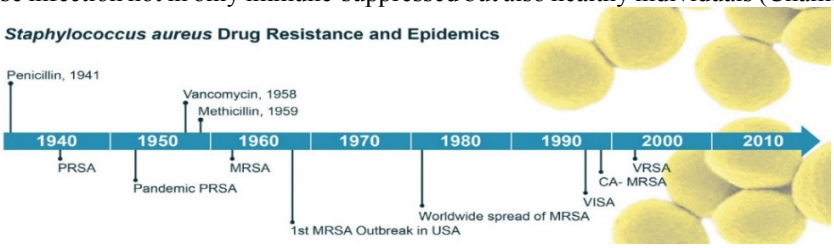

Fig. 1 Timeline of emergence of drug resistance in S. aureus. Source: McGuinness et al. (2017) (reproduced with copyright permission).

S. aureus seems to have developed β-lactam antibiotic resistance by receptor modification, as mentioned above. The altered penicillin-binding protein (PBP) is named PBP2a and is encoded by the *mecA* gene. PBP2a has low affinity to β-lactam antibiotics, which results in continuous peptidoglycan synthesis even in the presence of drugs (Berger-Bächi & Rohrer 2002). *mecA* is under regulation of the repressor MecI and transmembrane β-lactam-sensing MecR1. Transcription of both *mecA* and *mecR1* is repressed by MecI in normal conditions (absence of β-lactam antibiotic). In the presence of a β-lactam antibiotic, MecR1 is cleaved auto-catalytically, and the cytoplasm-localised region, the metalloprotease domain, becomes active. It cleaves MecI and then binds to the *mecA* operator region, promoting the transcription of *mecA* (Berger-Bächi & Rohrer 2002). The *mec* gene complex is located on a mobile genomic island called the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) (Ito et al. 2003).

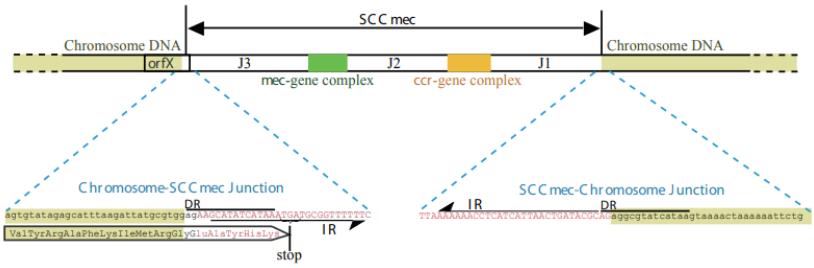

Fig. 2 SCCmec basic structure in chromosomal DNA. IR, inverted repeat; DR, direct repeat. Source: Hiramatsu et al. (2013) (reproduced with copyright permission).

Figure 2 presents the basic structure of SCC*mec* including the *mec* gene complex, the *ccr* gene complex encoding Cassette Chromosome Recombinase (CCR) that integrates/excises SCCmec into/out of the staphylococcal chromosome at a specific short sequence called SCC integration sites and three intergenic J regions containing possibly non-functional pseudogenes. The recognition sites are located within the direct repeats flanking the SCC. Two DRs are designated differently (DR-SCC and DR-B) as DR-SCC partially overlaps with the IR and remains in the SCC*mec* when the genomic island is cleaved out by CCR while DR-B is a special sequence downstream of an unknown function (*orfX*) which belongs to *S. aureus* chromosome (Katayama et al. 2000). These DRs can serve as recognition sites for other SCC*mecs*. The function of the IRs has not been clarified, however they may have a role in regulating *ccr* gene expression as Zhang et al. (2015) reported that replacement of IR leads to significantly increased expression of *ccrAB* genes. SCC*mec* elements have been classified into 11 types (I to XI) based on the nature of *mec* and *ccr* complexes, and further subtypes based on their J regions (Liu et al. 2016). Of these, SCCmec type II and III contain additional integrated drug resistance genes, e.g., plasmid pUB110 encoding resistance to a number of aminoglycosides, pI258 encoding resistance to penicillins and heavy metals, transposon Tn554 containing *ermA* encoding macrolide, lincosamide and streptogramin resistance, and ψTn554 encoding cadmium resistance (Deurenberg & Stobberingh 2008).

2.2 Vancomycin Intermediate *S. aureus* (VISA)

The glycopeptide antibiotic vancomycin was approved in 1958 and has become the treatment of choice for MRSA. For a long time, there was no reported vancomycin resistance in *S. aureus* (Fig. 1), hence the early reports of reduced susceptibility of *S. aureus* from Japan in 1997 elicited a major concern (Hiramatsu et al. 1997). According to the readjustment of Clinical and Laboratory Standard Institute (CLSI) in 2006, the vancomycin breakpoint has been reduced from ≤4 to ≤2 μg/mL and VISA are those isolates that have vancomycin MIC from 4 to 8 μg/mL and vancomycin-intermediate *S. aureus* (VRSA) are isolates with MIC ≥16 μg/mL. However, there is an intermediate phenotype designated

as heterogeneous VISA (hVISA). hVISA, derived from a single colony of *S. aureus,* has a majority of cells with little to no resistance to vancomycin and a small subpopulation with MIC equal to the VISA phenotype (4 to 8 μg /mL) (Howden et al. 2010). The resistance mechanisms of hVISA are not completely clear, however, reports have indicated that it could be epigenetic even rather than based on genetic mutations. Roch et al. (2014) reported that exposure of vancomycin-susceptible MRSA strains to β-lactam antibiotics triggered a hVISA phenotype, and no *de novo* mutations were found. On the other hand, genetic mutation has been illustrated to contribute to the VISA phenotype and some fundamental characteristics, including increased cell wall thickness, reduced cross-linking of peptidoglycan, and reduced autolytic activity, have been recognised (McGuinness et al. 2017). To correlate these to vancomycin resistance, the bacterial cell wall biosynthesis process and mechanism of action of vancomycin are relevant.

Briefly, peptidoglycan is produced by polymerization of its monomeric component (murein) synthesized in the cell then exported to the outside by lipid carriers. Two enzymes involved in the peptidoglycan biosynthesis are glycosyltransferase and trans-peptidase, also known as PBP. Glycosyltransferase links murein monomers to form nascent peptidoglycan chains, then PBP crosslinks these to the pre-existing peptidoglycan layer. During this step, PBP recognises and cuts the terminal D-alanine, then ligates the new terminal D-alanine to the tip of the pentaglycine from a pre-existing peptidoglycan chain. Vancomycin interferes by binding specifically to the D-Ala-D-Ala at the tip of pentapeptide stem, preventing PBP from carrying out the crosslinking. However, only about 80% of D-Ala-D-Ala is processed by PBP (Kim et al. 2000), and free D-Ala-D-Ala present in the cell wall can be bound by vancomycin, but this does not inhibit cell wall synthesis. Instead, the key target of vancomycin is the D-Ala-D-Ala on the newly formed peptidoglycan chain or the translocated murein monomers (Hiramatsu 2001). In VISA, thicker cell wall and reduced crosslinking mean significantly more free-D-Ala-D-Ala in the outer layers of cell wall that can trap vancomycin and protect the new chains and monomers. Reduced autolytic activity also contributes to the increased cell wall thickness and free D-Ala-D-Ala. Among the mutations that contribute to the development of VISA, some significant ones are genes encoding two-component regulatory systems including *graRS* and *walKR*. GraRS regulates transcription of genes involved in cell wall biosynthesis. The *graRS* mutations reduce vancomycin susceptibility (Meehl et al. 2007) and also affect the expression of global regulators which could be relevant to VISA development (Herbert et al. 2007). Downregulation of *walKR* operon by mutations or insertion of IS256 leads to enhanced cell wall synthesis and reduced autolysis (Utaida et al. 2003). However, VISA strains with higher resistance levels suffer significant fitness cost and often revert to lower resistance or full susceptibility (Gardete et al. 2012).

2.3 Vancomycin-resistant *Enterococcus* (VRE) and Vancomycin Resistant *S. aureus* (VRSA)

Enterococci are normal intestinal microbiota of healthy animals and humans. Among the > 50 different species identified so far, *Enterococcus faecalis* and *Enterococcus faecium* are the most dominant species causing infections in human (Gray et al. 1991). Enterococci are recognized as major nosocomial pathogens because of several reasons. Firstly, they are very persistent organisms; they can grow in 6.5% NaCl, in a wide range of pH and temperature (10°C to 45°C) (Huycke et al. 1998), and can survive on inanimate objects for weeks (Neely&Maley 2000). Secondly, they are intrinsically resistant to several antimicrobial compounds such as ampicillin, penicillin and most cephalosporins (Gold & Moellering Jr 1996). Finally, they are able to quickly develop resistance to even new antimicrobials such as linezolid, daptomycin and tigecycline (Bourgeois-Nicolaos et al. 2014, Kelesidis et al. 2011, Niebel et al. 2015). Of significant concern is the resistance of *Enterococcus* to glycopeptide antibiotics as the prevalence of VRE has been increasing recently. Enterococcal infections are the second leading cause of nosocomial infections in the USA, with *E. faecium* and *E. faecalis* accounting for 4.1% and 6.8%, respectively (Sievert et al. 2013). Since the first reports of VRE in 1980s (Leclercq et al. 1988), nine types based on phenotype and genotype have been characterized so far (Ahmed & Baptiste 2018).

The resistance mechanisms conferred by *van* operons all involve modification of the terminal D-Ala-D-Ala of the pentapeptide stem but there are two distinct types. One group (*vanA, vanB, vanD, vanM*) modifies D-Ala-D-Ala to D-Ala-D-Lac which decreases vancomycin binding affinity by about 1000 fold (Bugg et al. 1991), while the other group (*vanC, vanE, vanG, vanL, vanN*) produces D-Ala-D-Ser which decreases this affinity by about 7 fold (Reynolds & Courvalin 2005). Among the nine distinct *van* operons, *vanA, vanB* types are located on transposons, the most common in clinical isolates and intensively studied (Werner et al. 2008). This may explain why completely vancomycin resistant *S. aureus* is achieved by adopting the *vanA* operon on Tn*1546* from a VRE conjugative plasmid (Arthur et al. 1993). Thus, VRSA shares the same resistance mechanism with *vanA* VRE. The *vanA* operon has seven genes (*vanR, vanS, vanH, vanA, vanX, vanY, vanZ*) (Fig 3) and is regulated by a two-component sensor-regulator system encoded by *vanS* and *vanR*, respectively (Hong et al. 2008). *vanH, vanA* and *vanX* are important for the vancomycin resistant phenotype as together they change the D-Ala-D-Ala to D-Ala-D-Lac which has the aforementioned greatly reduced vancomycin affinity. *vanY* is a D,D-dipeptidase that cuts the D-Ala-D-Ala already bound to the tripeptide peptidoglycan precursor to form the stem pentapeptide, but the role of *vanZ* is not well defined (Gutmann et al. 1992).

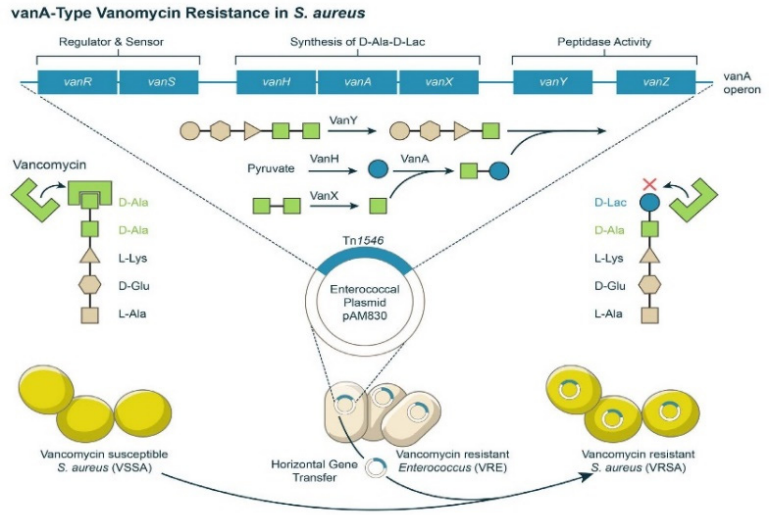

Fig. 3 Schematic illustrating the acquisition and mechanism of vanA-type vancomycin resistance. Source: McGuinness et al. (2017) (reproduced with copyright permission).

2.4 Multidrug-resistant (MDR) *P. aeruginosa*

P. aeruginosa is an aerobic, Gram-negative bacillus found ubiquitously in plants, soil and hospital water reservoirs. It has been recently reported as the sixth most common nosocomial pathogen and the second most common ventilator associated pneumonia in US hospitals (Weiner et al. 2016). According to CDC (https://gis.cdc.gov/grasp/PSA/index.html last accessed July, 2018), the overall percentage of *P. aeruginosa* that are resistant to fluoroquinolones, aminoglycosides, ceftazidime and piperacillin-tazobactam are 21.6, 9.7, 10.3, 10%, respectively. *P. aeruginosa* shows high-level of resistance due to its inherent tolerance to many classes of drugs and its ability to acquire resistance through mutations and horizontal gene transfer (Livermore 2002). As a result, multiple resistance mechanisms have been described, including β-lactamase production, Resistance-Nodulation-Division (RND) multidrug efflux pumps, porin alterations and target site modification (Strateva & Yordanov 2009).

Beta-lactamase production is one of the most prominent mechanisms of resistance employed by *P. aeruginosa.* Beta-lactamase can be the intrinsic AmpC, or acquired genes including OXA extended-spectrum beta-lactamases (ESBLs), or IMP or VIM class B metallo-beta-lactamases (MBLs) (Livermore & Woodford 2000). Normally, beta-lactamase resistance is an inducible process which involves the recycling pathway of peptidoglycan. The presence of β-lactam leads to an increase of peptidoglycan components in the intracellular environment, which are recycled by AmpD. When their amount exceeds the recycling capacity of AmpD, the excess fragments bind to and deactivate the AmpR repressor, and *ampC* expression starts (Juan et al. 2006). However, this system confers resistance to only some β-lactam antibiotics. It is the 'derepression' of *ampC* leading to hyper-production of AmpC through chromosomal mutations that confers resistance to some anti-pseudomonal agents such as ceftazidime and piperacillin-tazobactam (Juan et al. 2006). The second resistance mechanism is the efflux pump which can remove diverse types of drugs such as fluoroquinolones, chloramphenicol, β-lactams, tetracycline, macrolides, sulfonamides as well as many dyes and detergents (Poole 2001). The system consists of three components: the pump or RND protein, the outer membrane protein or exit portal and the membrane fusion protein or linker protein that connects them. The third mechanism is porin alterations. In *P. aeruginosa*, the porin OprD is accessible to carbapenems; hence its down-regulation leads to increased tolerance to carbapenems (Wolter & Lister 2013). Finally, *P. aeruginosa* achieves resistance against non-β-lactam antibiotics via antibiotic target modification. By modifying the 16S rRNA aminoglycoside-specific binding site via methylation, *P. aeruginosa* protects this site from being bound by the drug (Doi & Arakawa 2007). Mutations to topoisomerases II and IV gain fluoroquinolone resistance, as they are targets of this drug (Higgins et al. 2003).

3. Current and emerging treatments

Resistance development against antibiotics is a natural phenomenon which is part of the evolution and adaptation of bacteria. However, the emerging and alarming issue of antibiotic resistance is undoubtedly due to the misuse of these drugs over the years in humans, plants and animals. Hence, the need for development of new drugs from existing antibiotic classes, and novel drugs, is critical. The section below summarises the some new drugs from existing antibiotic classes developed in recent years and some potential novel treatments that are effective against the highlighted bacteria.

3.1 Beta-lactams

Cephalosporins are known for their broad-spectrum activity and favourable safety profiles, making them the most commonly prescribed antimicrobial class. The fifth generation cephalosporins, including ceftaroline and ceftobiprole, have a unique activity against MRSA and extended activity against Gram-negative resistant bacteria. Ceftaroline was approved by US Food and Drug Administration (FDA) in 2010 for treatment of community-acquired pneumonia (CAP) and acute bacterial skin and skin-structure infections (ABSSSIs). Ceftaroline is more potent against MDR Gram-positive bacteria including hVISA, VRSA and MRSA due to its effectiveness in inhibiting PBP2a (Poon et al. 2012). Similarly, ceftobiprole has high binding affinity for PBP2a. The two drugs, however, are labile to hydrolysis by class A, B and D ESBLs and carbapenemase (Queenan et al. 2007). A new promising cephalosporin is cefiderocol, which is under phase III trial. Cefiderocol has a catechol side chain which gives it a novel iron-binding property, allowing cefiderocol to be transported into bacterial cells through the siderophore system; thereafter, it binds to PBPs including 1a, 2 and 3 with PBP3 being the primary target (Page 2013). Cefiderocol is also stable against a number of beta-lactamases from Gram-negative bacteria including metallo-beta-lactamase, AmpC beta-lactamase and ESBLs (Ito et al. 2018).

3.2 Beta-lactam and beta-lactamase inhibitors

Beta-lactamase inhibitors (BLIs) are a potent strategy to overcome the resistance of bacteria, especially Gram-negative bacteria, against beta-lactam antibiotics. BLIs protect these antibiotics from being hydrolysed by beta-lactamases, by forming an irreversible covalent bond with the serine in the catalytic region of beta-lactamases and inactivating them. Following this is the slow fragmentation of the inhibitor molecule, but, interestingly, the enzyme adduct with inhibitor fragment has even longer half-life than the initial complex (Matagne et al 1993). This is also a limitation of BLIs as they are slowly eliminated and the beta-lactamases can regain their active state through hydrolysis of the adduct (Ehmann et al. 2012).The classical BLIs are typically more active toward class A serine beta-lactamases than class B and D serine beta-lactamases, but are not active to class B metallo-beta-lactamases (MBLs).

Avibactam used in combination with ceftazidime (a third generation cephalosporin) was approved by FDA in 2014 for treatment of complicated intra-abdominal infections (cIAIs) and complicated urinary tract infection (cUTIs). Avibactam is a new BLI with two key advance properties. First, avibactam has a wider inhibitory range against class A, C and some class D beta-lactamases, however it still does not have activity against MBLs. Second, the inhibition mechanism of avibactam is reversible which means hydrolysis does not take place and its activity is maintained (Falcone & Paterson 2016). Addition of avibactam significantly expands the activity of ceftazidime specifically against de-repressed AmpC *P. aeruginosa* strains, with about 80% of ceftazidime-resistant isolates exhibiting susceptibility to ceftazidime-avibactam (Sader et al. 2015). Another recently approved beta-lactam-BLI combination for the same indications is ceftolozane-tazobactam. This was reported as the most potent beta-lactam agent against *P. aeruginosa* by inhibiting 96.1% of isolates with resistance at MIC $\leq$ 4 μg/mL in a US surveillance study (Farrell et al. 2013). Ceftolozane is not affected by the *P. aeruginosa* efflux pump and OprD porin loss (Moyá et al. 2012) and, with its heavier side-chain, has higher stability to AmpC hydrolysis (van Duin & Bonomo 2016). Tazobactam does not enhance the activity of ceftolozane but, as a BLI, it further protects ceftolozane from ESBL phenotypes (Nguyen et al. 2018).

3.3 Glycopeptides

Glycopeptides have a limited activity spectrum, mainly against Gram-positive cocci. The first generation includes vancomycin approved in 1958 and teicoplanin approved in 1998 in Europe (Blaskovich et al. 2018), and their mechanism of action has been described above. Recently approved second-generation glycopeptides include telavancin and oritavancin. These are actually lipoglycopeptides and are characterized by greater potency, longer half-life and less potential for resistance development (Bassetti & Righi 2015). Telavancin, introduced by Theravance in 2009, is a derivative of vancomycin with attachment of a lipophilic tail to the vancosamine sugar (which enhances potency against Gram-positive pathogens) and a hydrophilic group on the aromatic ring of amino acid 7 (which improves ADME properties) (Leadbetter et al. 2004). Telavancin presents a rapid bactericidal property that could be due to a cooperative effect from binding to D-Ala-D-Ala of the pentapeptide terminal in nascent peptidoglycan together with membrane insertion of the hydrophobic tail, causing membrane leakage and depolarization without cell lysis (Higgins et al. 2005). Telavancin is less effective for *vanA* than *vanB* VRE because it induces expression of the *vanA* operon but not in *vanB* VRE (Hill et al. 2010). Oritavancin was also approved in 2014 for treatment of Gram-positive associated ABSSSI in adults. Unlike telavancin and dalbavancin, oritavancin shows potency against both *vanA* VRE and VRSA due to its multiple modes of action. It not only binds to the D-Ala-D-Ala termini to inhibit transglycosylation, but can also interrupt the transpeptidation process by binding to the pentaglycyl bridge of Lipid II. Furthermore, its hydrophobic side-chain can interact with the cell membrane, anchoring it to the cell and increasing its interaction with Lipid II. The interaction with the membrane also leads to its third mode of action, i.e., disruption of membrane integrity, causing depolarization and increased permeability (Zhanel et al. 2012).

3.4 Oxazolidinones

Oxazolidinones are synthetic antimicrobial agents developed over the past 30 years by many pharmaceutical companies (Shaw & Barbachyn 2011). Linezolid, the first generation of oxazolidinones, has shown activity against key Gram-positive pathogens including resistance strains such as MRSA, VRSA, and VRE. It is currently used to treat uncomplicated and complicated skin and skin structure infections (cSSSI) infections caused by VRE, and community associated pneumonia (CAP) and hospital associated pneumonia (HAP). The mechanism of activity involves protein synthesis inhibition. It binds to the V-domain of the 23S rRNA of the 50S subunit of ribosomes and interacts with the A-site of the peptidyl-transferase centre (PTC), preventing the incorporation of the incoming aminoacyl-tRNA and, thus, peptide elongation (Wilson et al. 2008).

The first widely reported reduced oxazolidinone susceptibility involved point mutations within 23S rRNA or L3 and L4 ribosomal proteins. However, since staphylococci and enterococci normally have four to six 23S rRNA gene copies, multiple mutations must be acquired before MICs reach the breakpoint. Ribosomal proteins L3 and L4 are located close to 23S rRNA, hence mutations in these proteins may also disturb the interactions between PTC and oxazolidinone (Rybak & Roberts 2015). The perhaps more worrisome resistance mechanism is conferred by the *cfr* gene which encodes the RNA methyltransferase, Cfr. This enzyme incorporates a second methyl group at A2503 of 23S rRNA which occupies a portion of the PTC binding site and leads to reduced affinity for linezolid (Arias et al. 2008). The *cfr* gene is often located on a transferable conjugative plasmid and, in fact, *cfr* has now been found in both Gram-negative and Gram-positive organisms (Shen et al. 2013). However, linezolid resistance is relatively rare (≤1%) across enterococci and staphylococci (Rybak & Roberts 2015). Tedizolid phosphate is the first of the second generation oxazolidinones approved by US FDA in 2014. It has higher antimicrobial potency than linezolid, favourable pharmacokinetics and lower rates of adverse effects. One more advanced property of tedizolid over linezolid is that its activity is less affected by resistance mechanisms than linezolid and is not affected by Cfr methylation (Locke et al. 2014).

3.5 Tetracyclines

Tetracyclines mechanism of action involves inhibition of protein synthesis by binding to the 16S rRNA of the 30S ribosomal subunit and blocking the attachment of aminoacyl tRNA to the A site, preventing peptide elongation (Beale 2011). This class of drugs has broad-spectrum antibacterial activity and is used widely not only in human and animals, but also in agriculture. This led to the development of various resistance mechanisms and reduced usage for indications such as respiratory, intestinal and urinary tract infections (Thaker et al. 2010). Although semisynthetic processes play an important role in the development of tetracyclines, they also have some limitations in the modification of the functional groups at specific carbons in the 4 core rings of this drug. This issue was resolved by a new total synthesis method and many new tetracycline candidates have been produced; noticeable among these is the novel fluorocycline, eravacycline (Ronn et al. 2013, Xiao et al. 2012). Eravacycline has gone through all clinical trial phases and is currently in preregistration for treatment of complicated intra-abdominal infections (Tetraphase Pharmaceuticals 2018, http://ir.tphase.com/news-releases/news-release-details/tetraphase-pharmaceuticals-announces-fda-acceptance-filing-its). Eravacycline has a wide range of activity against both Gram-positive and Gram-negative pathogens including MRSA, VRSA, VRE and ESBL-producing and carbapenemase-producing Enterobacteriaceae (Bassetti & Righi 2014). However, it is not active against *P. aeruginosa.* Four tetracycline-specific resistance mechanisms are known so far, i.e., tetracycline-specific efflux pumps, ribosomal protection proteins (RPPs), rRNA mutations and drug degradation (Nguyen et al. 2014), of which the first two are most prevalent. The efflux pumps confer resistance to tetracycline, inconsistent resistance to minocycline, and none to tigecycline and eravacycline (Chopra & Roberts 2001). The pumps are located in the cell membrane of both Gram-positive and Gram-negative bacteria. RPPs weaken the interactions between tetracycline and its binding site in the ribosome. The most prevalent RPPs are TetO and TetM identified in clinical isolates of Gram-negative and Gram-positive bacteria (Thaker et al. Wright 2010). In *E. coli* expressing *tetM*, the activity of eravacycline remains unaffected while the MIC of tigecycline increases (Grossman et al. 2012). For all enterococci, streptococci and staphylococci, eravacycline is generally 2-4 fold more potent than tigecycline and this holds for most Gram-negative aerobic bacteria (Zhanel et al. 2016).

3.6 Emerging and potential treatments.

Besides the developments of the conventional antibiotics, some emerging treatments with great potential are also worth mentioning. Treatments that will be briefly discussed in this part include antimicrobial peptides, antivirulence approaches and antibiotic potentiators.

Antimicrobial peptides (AMPs), also known as host defence peptides, have existed in plants and animals for millions of years for defense against pathogens (Wang 2017). Interestingly, some AMPs are produced by microorganisms and these are used mainly for killing of other competitors or to protect their hosts (Quinn et al. 2012).In general, AMPs are short cationic and amphipathic peptides (<50 residues). AMPs preference for bacterial over eukaryotic cells is due to the differences in their membrane composition. Bacterial outer membranes are negatively charged as they contain anionic lipids (e.g. phosphatidylglycerol, cardiolipin) while eukaryotic membranes are rich of zwitterionic (neutral) lipids (e.g. phosphatidylcholine, sphingomyelin) and also cholesterol (Yeaman & Yount 2003). Negatively charge bacterial

membranes are more attractive to cationic AMPs due to electrostatic effect. And after adsorption to bacterial membrane the hydrophobic part of AMPs interacts with the hydrophobic core of the membrane and this helps AMPs to insert into the membrane (Yeaman & Yount 2003). Initially, membrane disruption was believed to be the mechanisms of AMPs action. However, it has been shown that AMPs can kill pathogen without cell lysis. This means some AMPs can translocate through membrane and target intracellular components such as protein synthesis and DNA, RNA or even organelles (Teixeira et al. 2012). In fact, AMPs target multiple targets of the pathogens and have been described as "dirty drugs" (Peschel & Sahl 2006). AMPs have a high potential to become new antimicrobial drug as they possess a number of advanced properties such as: a broad activity spectrum, low rate of resistance development (due to their multiple-target mode of action), synergy with conventional antibiotics and indirect killing of bacteria through modulation of immune system or ability to neutralize endotoxins (Hancock & Diamond 2000, Yeaman & Yount 2003). Even though, there are still some weaknesses that limited the amount of approved AMPs such as haemolytic activity, host toxicity, low in vivo stability against proteases and sometimes cost of production (Boto et al. 2018), we can still be optimistic about the future of AMPs as more effective strategies have been employed to optimise AMPs and to explore new AMPs (Boto et al. 2018). One very fascinating AMP that was discovered recently is teixobactin by Ling et al. (2015) from an uncultured bacteria temporarily named *Eleftheria terrae.* Teixobactin has shown to be effective against some drug resistant gram-negative bacteria including MRSA (MIC 0.25 μg/mL), VRE (MIC 0.5 μg/mL). Teixobactin presents the high potency because it targets the crucial cell wall synthesis process of bacteria, however unlike other conventional antibiotics, teixobactin appears to interact with non-protein compartment of both peptidoglycan and teichoic acid precursors namely lipid II and III which explained why no resistance detected in the study (Ling et al. 2015). The more important thing is the iChip technique introduced by Nichols et al. (2010) which has been used for the identification of teixobactin opened a new window for AMPs discovery as around 99% of the bacteria in environment is still unculturable (Lewis 2013).

On another point of view, having a broad spectrum of activity may not be a good idea. The main problem with this is broad spectrum antibiotics not only kill pathogenic bacteria but also kill many of beneficial and commensal bacteria which are part of our microbiota. Some of these also have important roles in human health such as increasing metabolic capabilities, promoting immune system development and preventing colonization of pathogenic bacteria (Cho & Blaser 2012). The mass killing of conventional antibiotic creates a high evolutionary selective pressure for development of resistance which is the main reason for quick development of antibiotic resistance in conventional antibiotics. The new strategy which can overcome these problems is antivirulence drugs because these only target the virulence factors of pathogens which commensal bacteria lack. Number of virulence factor is large and diverse generally include adhesins, secretory systems, toxins, siderophores, biofilm promotion factors, quorum sensing and so on. In CA-MRSA, α-toxin is one of the major virulence factors that is highly expressed. Monoclonal antibody (mAb) MEDI4893 inhibits the oligomerization and interaction of α-toxin with its cognate receptor and hence MEDI4893 has displayed protective effect against *S. aureus* in many animal models (Dickey et al. 2017). It is currently under phase II trial for nosocomial pneumonia (Adis Insight 2018, https://adisinsight.springer.com/drugs/800017995). M64 blocks quorum sensing across various *P. aeruginosa* clinical isolates and promotes survival in mouse burn and lung models (Starkey et al. 2014). There are however still some disadvantages regarding this approach such as more labour and higher cost, multiple antivirulence drugs may be required for a disease, lower therapeutic outcome as it does not kill pathogens.

The final strategy for combating antibiotic resistance is the use of antibiotic potentiators. These compounds can function by either potentiating the effectiveness of antibiotics or reversing resistance mechanism of resistant bacteria. Beta-lactamase inhibitors mentioned above also belong to this category. Plant natural low molecular weight (MW) metabolites appeared to be promising potentiators. Epigallocatechingallate (EGCg), a polyphenol in plant low MW metabolites, has shown great potential. EGCg used in combination with beta-lactams including oxacillin, ampicillin, methicillin, benzylpenicillin and cephalexin reversed the resistance of MRSA (Zhao et al. 2001). The authors claimed that EGCg synergizes with beta-lactam due to its ability to bind directly to peptidoglycan and disrupt the cell wall integrity. Combination of EGCg with penicillin restored antibacterial activity of penicillin against penicillinase-producing *S. aureus* by inhibiting penicillinase activity (Zhao et al. 2002). Efflux pump inhibitors (EPIs) is also a major area of research in this field, however there is no EPIs approved for clinical use due to some problems such as low efficacy, low specificity which leads to off-target effects. Fleeman et al. (2018) recently has reported a novel polyamine scaffold with potent efflux pump inhibition activity against MDR bacteria. Using a high-throughput library screening, the authors have identified some molecules with high specificity, no toxicity toward mammalian cells. Combination treatments of these compounds with tetracycline significantly enhance bactericidal activity of the drug and potentiate tetracycline antibiofilm activity against *P. aeruginosa*. Even certain types of antivirulence drugs can be used as potentiators. For example, MEDI3902, a bispecific antibody which targets surface-associated virulence factors including PcrV (Type III secretion system subunit) and PsI (exopolysaccharide), has shown synergetic effect with multiple antibiotic classes (Di Giandomenico et al. 2014).

4. Conclusions

At present, our battle with bacterial infections is becoming dire with more MDR-bacteria and limited drug choices. However, there should be optimism about the future as variations of conventional and novel drugs have shown great

potential and are on their way to the market. Besides, there are some novel strategies that are worth considering that have not been covered in this review such as the use of bacteriaphages and lysins, therapeutic antibodies and vaccines against bacterial infections which add even more future treatment options. Nevertheless, we should keep in mind that resistance development is a natural process for bacterial survival and it is anticiapted that resistance will enentually appear to any new drugs. As long as we improve the way that antibiotics are used and prescribed, we should be able to maintain some control of the situation.

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