Chapter

Strategic Role Players of Important Antimicrobial-Resistant Pathogens

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Abstract

Over the years, tireless efforts of the concerned scientists have produced various new therapeutics and methods for the treatment of bacterial infections. However, despite the vast regimen of modern antibiotics being corroborated, the diseases caused by the Gram-positive and -negative pathogens has become untreatable, mainly due to the constantly evolving threat of antimicrobial resistance (AMR), thereby leading to huge morbidity and mortality. Moreover, shortage of efficient therapies, lack of successful prevention strategies and availability of only a few effective antibiotics urgently necessitated the development of novel therapeutics and alternative antimicrobial treatments. These developments have been based on the molecular mechanisms of resistance posed by the pathogens during their interactions with the host. Herein, we collate four essential bacterial components like chaperones, efflux pumps, two-component systems and biofilms which can present challenges for the most coveted control of infection. Essentially, we discuss the current knowledge status of these components to provide insight into the complex regulation of virulence and resistance for some medically important multidrug-resistant (MDR) pathogens. This will help the future scientists to clearly focus on some specific proteins to be targeted by against the available class of drugs and/or antibiotics with the broader perspective to develop novel antimicrobial agents.

Keywords: antimicrobial resistance, biofilms, chaperones, efflux pumps, multidrug resistance, two-component systems

1. Introduction

Bacterial infections have been threatening human population since time immemorial. Being one of the leading causes of morbidity and mortality (**Figure 1**), the latest global rise in antibiotic resistance threatens to undo decades of progress in treating such bacterial infectious diseases caused by the pathogens. In fact, multidrug resistance (MDR) conferred by Gram-positive and -negative bacteria is difficult to treat and may even be, untreatable with conventional antibiotics. The case has turned out to be so serious that many of these microorganisms are at least resistant to a single drug regimen while several are moving from developing MDR to extensively and total drug resistance, referred to as XDR and TDR, respectively.

All the aforementioned classes of resistance, namely MDR, XDR and TDR, commonly referred to as antimicrobial resistance (AMR), has been conferred the main cause for the second leading global disease burden of bacterial infection in the twenty-first century, as reported by WHO [1]. Importantly, the development of

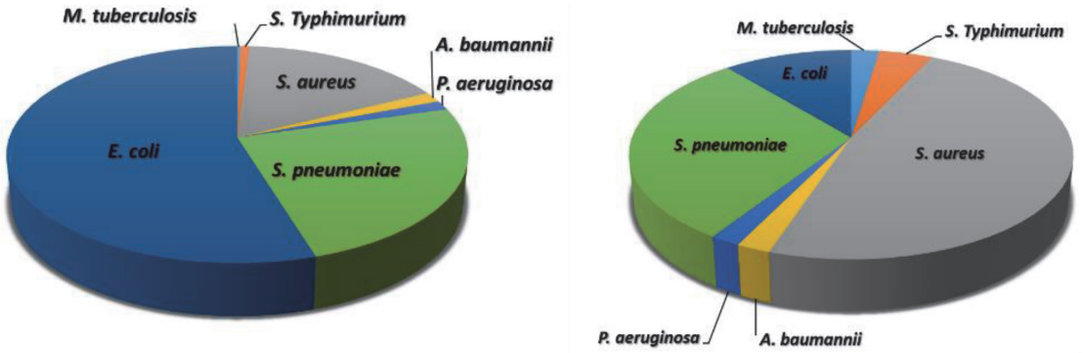

Figure 1.
Rates of infection (left) vs. mortality (right) statistics as per National Center for Health Statistics (CDC), 2017.

antibiotics has directly influenced the initial resistance caused by using newer agents. Moreover, the discovery of new antibiotic classes is reported to be void since 1987, when lipopeptides was the last class introduced (**Figure 2**) [2]. Thus, it has become increasingly difficult to find therapeutic options to treat organisms developing AMR, such as *Acinetobacter baumannii, Proteus mirabilis and Pseudomonas aeruginosa* [2]. Nevertheless, antimicrobials have had a significant positive effect on the administration of irresistible infections and have become a basic component of all perspectives of modern healthcare.

The rise of AMR development has become a serious concern more than what can be even perceived. This is potentiated by different facts ranging from adverse effects of existing antibiotics and consequent re-purposing and/or chemical modification or their withdrawal leading to the sparing usage of new ones due to resistance concerns and ultimately a shortage in the development of new antibiotics [3–5]. Moreover, environments of hospitals and other health care systems as well as social communities and advanced transport systems have enabled the spread of AMR easier and faster [6]. This is evidenced by a recent increase in the carbapenem resistance (e.g. meropenem) due to the presence of carbapenemase, a.k.a. New Delhi

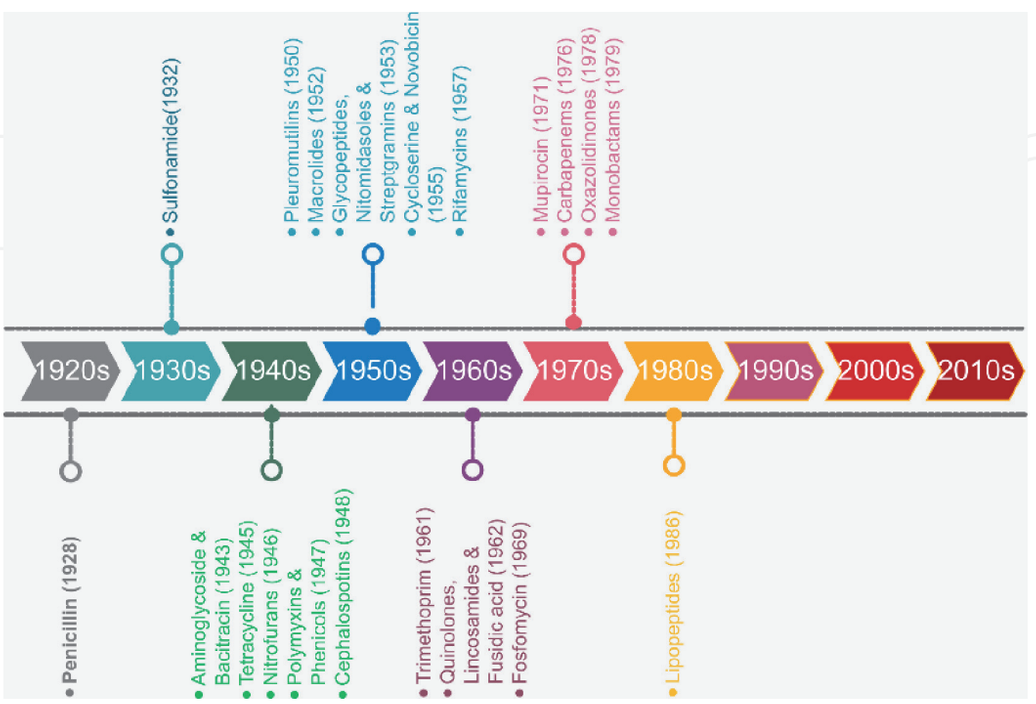

Figure 2.
The timeline of the development of different antibiotic classes.

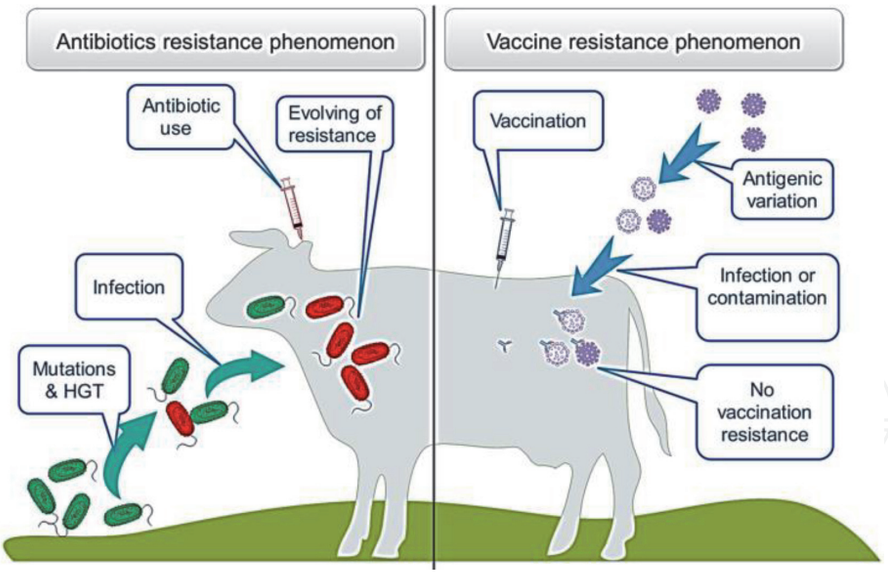

Figure 3.
Antibiotic vs. vaccine-resistant phenomenon. HGT represents horizontal gene transfer, green and red colored cells denote antibiotic-sensitive and -resistant bacteria, respectively.

metallo-β-lactamase-1 (NDM-1) in various Enterobacterales isolates [7]. Initially reported to have found in a patient in Sweden in 2008 who had originated from India, such cases were found later in UK patients having either travel or ancestral history from the Indian subcontinent [6]. A variation of no such travel or hospital contacts, for patients harboring NDM-1, was also reported by 2011 [8], along with drinking water and sewage samples containing a range of NDM-1 harboring bacteria (e.g. *Shigella boydii and Vibrio cholerae*) [9] thereby proving that AMR development varies within organism and with the mechanism of transfer of mobile resistance elements between species (**Figure 3**). Again, some vaccine resistance phenomenon has added on to activities while researchers are aiming to produce advanced vaccines through recombinant DNA technology, keeping in mind the utility of vaccines over antibiotics (**Figure 3**).

2. The causes

AMR is exhibited when a microorganism survives in the presence of an antibiotic concentration that is generally adequate to prevent or stop its growth. Thus, in clinical terms "prone" and "resistant" are generally used to infer the efficacy or failure of medical therapy, respectively [10]. Moreover, the microbes can either be inherently resistant to an antibiotic or develop resistance after their exposure to incorrect and/or insufficient dosage prescription. This is commonly the case for patients routinely communicating with hospital settings thereby having gradually increased resistance to frequently used antibiotics. For these cases of hospital-acquired infections (HAI), certain bacteria develop drug-resistant strains through natural selection mechanism which promotes the persistence of bacterial strains having acquired some mutation [11]. However, the increased profile of these pathogens with AMR varies, even though they arise from similar causes.

AMR resistance may evolve as a mechanistic consequence of gene mutation or direct gene transfer, the latter being also known as horizontal gene transfer (HGT). Of these two, HGT helps to acquire new resistance genes and virulence determinants through a multitude of mechanisms including conjugation, transduction or transformation among related and/or non-related species [10]. This phenomenon is

commonly associated with bacterial adaptation to new niches or lifestyles and has an impact on the development of its genomic content. Again, HGT, with the help of mobile genetic elements (MGEs) like transposons, has been reported to have conferred resistance to a broad range of antibiotics, particularly toward new ones. Moreover, transmissible plasmids and phages often bear genes that confer antibiotic resistance to one or more distinct antibiotics and facilitate their transfer across different genera. Such evolution essentially underpins the survival of the developing MDR strains and may be a major reason for the global outbreaks.

3. The effectors

Besides the emerging species of bacteria exhibiting MDR, namely *Salmonella enterica*, *Mycobacterium tuberculosis* and *P. mirabilis*, other common species of MDR bacteria responsible for two-thirds of all HAIs are defined by the acronym ESKAPE to denote the six pathogens namely, *Enterococcus faecium, S. aureus, Klebsiella pneumoniae, A. baumannii, P. aeruginosa* and *Enterobacter* spp. [12]. These are easily distinguishable from other pathogens due to their enhanced resistance to frequently used antibiotics such as penicillin, vancomycin, carbapenems and more. One of the common resistance mechanisms involves enzyme production that alters the antibiotic target sites and results in no binding activity with efflux pumps [13]. Efflux pumps are the characteristics of the Gram-negative bacterial membrane that enables them to constantly pump out foreign materials, including antibiotics, such that the intracellular milieu does not have sufficiently elevated drug concentration to make the effect [13]. Moreover, biofilms are a combination of different microbial and polymer groups that protect the bacteria from antibiotic therapy by acting as a biological barrier [13].

3.1 *Salmonella enterica*

Human infections due to *S. enterica*, a bacterial pathogen, constitute significant food borne disease burdens of blood stream associated with a high mortality ratio throughout the world [14, 15]. *S. enterica* are the Gram-negative facultative anaerobe that belongs to the family Enterobacteriaceae. From over 2,500 strain types, the strain *S. enterica serovar* Typhi causes the typhoid fever [16]. Infections with *Salmonella* in humans typically range from non-typhoidal salmonella (NTS) to typhoidal fever, which can be life-threatening. Additionally, the resistant serovars causing enteric fever, namely, Typhi, Paratyphi A, B, or C are broadly referred to as typhoidal *Salmonella* serovars [14]. However, these are highly adapted to the human host that is used as their exclusive reservoir [17].

The initial AMR acquired by *Salmonella* was to the first-line drugs such as ampicillin, chloramphenicol and sulfamethoxazole. The AMR mechanisms in *S.* Typhi include drug inactivation, target site modification and active efflux, which might be chromosomal or plasmid-mediated [18]. In fact, the resistance of *Salmonella* and pathogenic *E. coli* along with other Gram-negative bacteria, against antibiotic and non-antibiotic compounds, is related to efficient efflux pumps, which reduces the intracellular concentration of such compounds [19, 20]. The occurrence of plasmid-mediated antibiotic resistance to fluoroquinolones has recently been recorded and referred to a single point mutation in the topoisomerase gene gyrA, encoding DNA gyrase. Moreover, pathogenic *Salmonella* uses the two-component systems (TCS) namely, PhoPQ, PmrAB and Rcs regulatory system for lipopolysaccharide (LPS) modification and increases the resistance toward host human AMPs [18], which could help it to survive *in vivo* and develop the disease [21]. The lack of

such systems in the eukaryotic host made them eligible to be targeted by anti-virulence compounds. This strategy was rendered successful during selective active site inhibition of PhoQ autokinase activity by Quinazoline [22]. Furthermore, for cells lacking an RNA chaperone, known as bacterial cold shock proteins (CSPs), high levels of porin genes *viz*. *omp*D, *omp*F, and *omp*C resulted in increased cell membrane permeability in response to bile salt stress [23]. This finding highlights on the importance of the chaperone protein in the maintenance of the membrane integrity and selective permeability.

3.2 *Proteus mirabilis*

P. mirabilis, the Gram-negative uropathogenic bacteria and a member of the Enterobacteriaceae family, is developing MDR to antibiotics and biofilm formation. This may trigger significant complications in patients with long-term catheters or urinary tract infections (UTI) [24, 25]. *Salmonella* genomic island 1, an integrative mobilizable component of multidrug-resistant *S.* Typhimurium, was recently identified in a remarkably high proportion of *P. mirabilis* clinical isolates from France, indicating its involvement in the spread of this MDR element [26]. *P. mirabilis* is susceptible to aminoglycosides, fluoroquinolones, sulfamethoxazole and β-lactams, but resistant to tetracycline and nitrofurantoin [27]. This enhanced resistance to antimicrobial agents has resulted not only in modifications to antimicrobial therapies, but also in poor diagnosis and increased mortality rate of nosocomial infections [28]. Astonishingly, in 2016, a new isolate of *P. mirabilis*, from diabetic ulcer patient, have shown a remarkable resistance to silver nanoparticles, spreading the alarm of resistance to even include metallic nanoparticles like silver [29]. Moreover, the efflux pumps (EPs) also play important role in *P. mirabilis* drug resistance, as exemplified of the increased cell permeability of the EP inhibitor Phenylalanine-Arginine Beta-Naphthylamide (PAβN), thereby making it more susceptible to acetylsalicylic acid [30].

3.3 *Acinetobacter baumannii*

A. baumannii are the most successful Gram-negative opportunistic nosocomial pathogens responsible for hospital-acquired infections (HAI) in intensive care units. The WHO has stated *A. baumannii* to be one of the most serious ESKAPE organisms that have effectively escaped the effects of antibiotics [31]. Several resistance mechanisms are known, including target modifications, multidrug efflux pumps, enzymatic degradation or modification of drugs and permeability defects besides some other uncategorized ones [31]. *A. baumannii* strain isolates are resistant to cephalosporins and penicillins, including inhibitory combinations of aminoglycosides and fluoroquinolones [32]. Moreover, some *A. baumannii* strains can acquire families of EP from other species and new β-lactamases to improve the resistance of β-lactam antibiotics [11]. Furthermore, *A. baumannii* clinical isolates are reported to be resistant to colistin developed due to a modification of the lipid A component of the lipopolysaccharide outer membrane. The modification is mediated by the TCS PmrAB and a mutation of the LpxA/C/D gene [33]. Such resistance mechanism through LPS modification brings out the importance of the TCS in *A. baumannii*.

3.4 *Staphylococcus aureus*

S. aureus is a major Gram-positive pathogen, both within the hospital settings and environmental communities and reported to be prone to nearly any antibiotic

ever produced [34]. Such multiple antibiotics resistance has developed by acquiring MGE through HGT. This results in mutations that alter drug binding sites on molecular targets leading to an increase in the expression of endogenous efflux pumps. These resistant strains fight antibiotics by deactivating β-lactam binding proteins [11]. Due to its increasing antibiotic resistance to penicillin and methicillin, the bacteria remain a growing pandemic through mechanisms including HGT and antibiotic alterations [35]. Moreover, *S. aureus* is not far from the Gram-negative bacteria which are resistant to antibiotics mediated by TCS. Thus, the TCS $VanR_AS_A$ regulates the necessary mechanism of resistance in vancomycin resistance *S. aureus* (VRSA) [36]. Again, the EPs from *S. aureus* have been categorized recently in six different diverse groups. They were found to be either chromosomal or extrachromosomal except *qac*A/B and *smr* which were found only on the studied plasmid samples [37].

3.5 *Pseudomonas aeruginosa*

P. aeruginosa, the Gram-negative nosocomial pathogen, is considered as an epitome of AMR due to its major involvement in causing chronic and nosocomial diseases. This high rate of resistance is directly related to their various inherent resistance mechanisms expressed, including the down-regulation of porin manufacturing system (carbapenems and cefepime), overexpression of efflux pumps (carbapenems) or production of other beta-lactamases besides the high production of AmpC beta-lactamase. The most frequently administered antipseudomonal antibiotics are aminoglycosides, fluoroquinolones and β-lactams that are susceptible to the known resistance mechanisms in *P. aeruginosa*. Its mutants, with upregulated EPs, have been reported that makes it difficult to find an effective antibiotic [38]. Moreover, only inhibition of the EP, in the recent clinical MDR isolates, has almost no effect in increasing susceptibility toward the tested antibiotics [39]. However, inhibition of histidine kinases (HKs), a part of TCS, using benzothiazole-based HK inhibitors, resulted in a reduced production of molecules which are linked to quorum-sensing and redox-balance. It also showed reduced motility and attachment ability, rendering it to be less virulence [40].

3.6 *Mycobacterium tuberculosis*

Tuberculosis (TB) poses serious global health crisis as an important chronic infectious disease caused by strains of *M. tuberculosis* (MTB). It is an extremely dangerous human pathogen that infects one-third of the world's population and causes almost two million fatalities each year [41]. Besides that, the total number of cases have been still increasing, due to strains of MTB being resistant to first-line drug therapy [41]. This involves resistance to the two most powerful anti-TB drugs, rifampicin and isoniazid, thereby evoking the title of multidrug resistance TB (MDR-TB). The existence of even more resistant MTB strains has been described as extreme drug-resistant (XDR)-TB, which shows resistance against the injectable second line drugs such as kanamycin, amikacin or capreomycin [42]. A more alarming situation has arisen with the depiction of MTB strains showing resistance to all antibiotics available for testing, with the species being termed as total drug resistant (TDR)-TB [43]. Therefore, the early onset of detection and prevention of MDR-TB, will enable the therapeutic treatment to reduce the spread of infection. Thus, a better understanding of the mechanisms of action of anti-TB drugs will facilitate the development of new drug targets aimed at improving outcomes from diseased patients [44]. In fact, it has been found that MprA, part of TCS MprAB.

along with other TCS, namely, TrcRS, control the expression of antibiotic resistant related β-propeller gene Rv1057 [45, 46]. The roles of chaperone(s) in the resistance of *M. tuberculosis* are yet to be declared.

3.7 *Klebsiella pneumoniae*

K. pneumoniae is a Gram-negative hospital-acquired pathogen causing nosocomial pneumonia and urinary tract infections. The increased incidence of carbapenemase-producing and thus, carbapenem resistant *K. pneumoniae* (CRKP), has posed a major threat to global human health. Diseases caused by CRKP were treated successfully in combination therapies of antimicrobial agents [47]. It has been reported that tigecycline and the polymyxins (polymyxin B or colistin) showed variable susceptibilities to treat infections caused by CRKP [4]. This has led to the emergence of CRKP, against which there are very few antibiotics in development that can treat the infections [11]. Incidentally, the minimum inhibitory concentration (MIC) of eravacycline has been increased as a consequence of increased expression of two EP complexes OqxAB and MacAB in *K. pneumoniae* [48], which suggests their contribution to resistance against this antibiotic.

3.8 *Enterococcus faecium*

Generally associated with HAI in immunocompromised patients, *E. faecium* is a Gram-positive bacterium, often showing resistance to β-lactam antibiotics, including penicillin and other antibiotics of last resort. Reportedly, there has also been an increase in vancomycin-resistant enterococci (VRE) strains, exhibiting resistance to vancomycin-A [11]. These VRE strains show an ability to produce and share their resistance through HGT, as well as code for virulence factors that regulate phenotypes. These virulence phenotypes differ from the wild types in producing thicker biofilms for development in a variety of environments, including medical devices such as urinary catheters and cardiovascular prosthetic valves [14]. The thicker biofilms function as a "mechanical and biochemical shield" that protects the bacteria from antibiotics and is the most efficient protective system against bacterial treatment [13]. In fact, the intensive use of antibiotics in animal rearing resulted in the development of resistance in *E. faecium* [49]. Moreover, recently, a study identified few new antibiotics resistance genes related to EP, namely, *optrA* and *poxtA* besides the new gene *cfr*-like variant in *E. faecium* [50]. Earlier, the expression of the EP proteins, EfrAB, has been shown to be increased upon halving the MIC of gentamicin and got lowered upon the addition of 3 mM EDTA [51]. Furthermore, TCS like ChtSR has been found to be responsible for chlorhexidine tolerance in MDR *E. faecium*, upon testing by targeted deletion mutation of *cht*R and *cht*S genes [52]. Again, the TCS CroRS was reported to be crucial in resistance to cell wall antibiotics in *E. faecium* [53].

3.9 *Enterobacter*

Enterobacter are Gram-negative bacterial species which trigger UTI and blood diseases. They show resistance against different drug therapies, thus, requiring the development of new and efficient antibiotic treatments [54]. In fact, colistin and tigecycline are, only two of the antibiotics, presently being used as medication while no other feasible antibiotics are apparently being developed. Other most commonly reported antimicrobials in *Enterobacter* infections are aminoglycosides, cephalosporins, carbapenems and fluoroquinolones. Moreover, in some species, a 5- to 300-fold rise in the MIC was reported when subjected to several gradually increasing

benzalkonium chloride concentrations [55]. In fact, the EP protein, SugE, in *Enterobacter cloacae,* which is a member of small multidrug resistance (SMR) protein family, has been found to be responsible for resistance against toxic compounds such as cetylpyridinium chloride and benzalkonium chloride [56]. Another EP protein of the resistance nodulation cell division (RND) category, AcrB, has also been found to be very essential in the pathogenicity and antibiotics resistance of *E. cloacae* [57, 58].

4. The role players

Several health interventions have been proposed as alternatives to current antibiotic therapy and prevent the resistant mechanisms of which, the development of new drug classes, use of vaccines or other therapeutic strategies are noteworthy (**Figure 4**) [59]. In fact, using computational approaches, certain proteins and/or phenotypes, having plausible involvement in antibiotic resistance, are proposed [60–64] as discussed below.

4.1 Chaperones

Bacterial chaperones like DnaK, belonging to the heat shock proteins (Hsp)70 family, are produced by cells in response to exposure to stressful conditions [65]. The interaction between the two domains of such Hsp70, namely ATPase and the substrate-binding domain, triggers the chaperone-based activity of DnaK which are also enhanced by the co-chaperone such as DnaJ (Hsp40 family) and chaperone GroE (Hsp60 family) [66]. DnaK acts on unfolded and partially folded protein chains by binding and controlling their configuration [67].

Besides stress response, DnaK plays a significant housekeeping role in maintaining normal bacterial cellular growth and homeostasis [68]. Thus, any

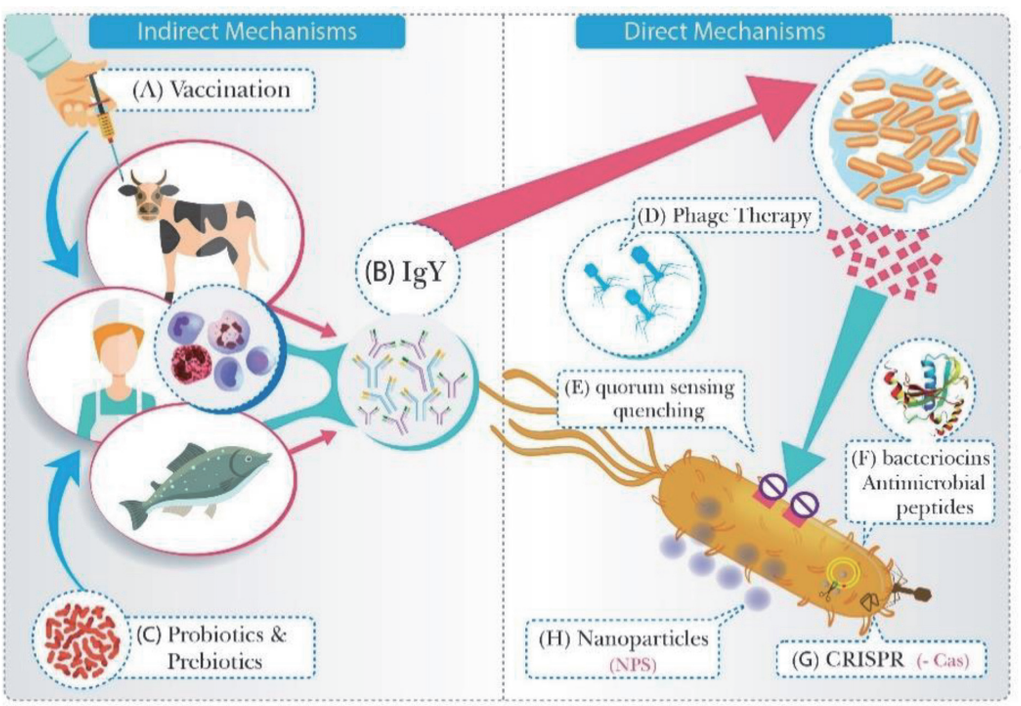

Figure 4.
Alternative strategies to combat antimicrobial resistance and their mechanisms of action.

alterations in the *dnaK* gene reduce the growth of bacteria within the host [69]. In fact, during infection, bacteria activate their heat shock genes like DnaK to protect their cellular machinery from the consequently activated host immune system for defense mechanisms and thereby strengthen their virulence strategy [69]. This phenomenon, thus, provides an insight into structural mechanism of DnaK, leading to misfolding and its role in controlling protein activity contributing to the pathogenicity of multidrug-resistant bacteria, such as the opportunistic human pathogen *A. baumannii* [70]. In fact, DnaK mutants showed decreased viability and improved susceptibility under strained circumstances during systemic infection as reported for *dnaK* mutants of *S. aureus* with increased sensitivity to oxacillin and methicillin [71] and *dnaK/dnaJ* mutants of *E. coli* having increased sensitivity to fluoroquinolones [72].

4.2 Efflux pumps

Antibiotic resistance can be triggered, in MDR bacteria, by four discrete mechanisms viz. target modification, reduced permeability and improved efflux, drug inactivation and drug extrusion by the multidrug efflux pumps (EP) [73]. Due to their poly-substrate specificity, besides having the potential to expel a broad variety of antibiotics, these EP also manage the development of other resistance mechanisms by decreasing intracellular antibiotics concentration and stimulating mutation accumulation [73]. Consequently, over-expression of multidrug EP is involved with clinically related antibiotic resistance. Thus, there has been increasing evidence of EP having biochemical functions in bacteria along with their appearance under strict regulations in response to some physiological and environmental signals [73]. Hence, a systematic knowledge of EP is important for the development of EP inhibitors as promising AMR intervention strategies.

EP are present in almost all bacterial species involved in AMR. They can be located on plasmids or chromosomes that encode this class of proteins. The five families of bacterial EP, found to be involved in MDR, are the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the ATP-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family, and the resistance-nodulation-division (RND) family, based on their composition, energy sources and substrates used [73]. Importantly, only RND superfamily is found in Gram-negative bacteria due to its structure containing tripartite complex and the efflux systems of the other four families are widely distributed in both Gram-positive and -negative bacteria. These EP can be either single or multiple-component transporters depending on their specific classes. They comprise both an inner and an outer membrane transporter, like the RND type. It has been found that RND family pumps are frequently associated with therapeutically important bacterial resistance such as AcrB in *S.* Typhimurium and *E. coli* and MexB in *P. aeruginosa* owing to their tripartite complex, enabling various drugs to be immediately extruded from cytoplasm to outside the bacterial cells [74].

In fact, antibiotics such as fluoroquinolone, tetracycline, rifampin, novobiocin, chloramphenicol and B-lactams were used to analyze the substrate profile of housekeeping efflux system AcrAB-TolC in *E. coli* [74]. Similarly, the *S.* Typhimurium AcrAB-TolC efflux system was also capable of expelling various antibacterial agents such as tetracycline, quinolones and chloramphenicol [75, 76]. The two RND efflux pumps, MexAB-OprM and MexXY-OprM, homolog to AcrAB-TolC system in *E. coli*, are also expressed in *P. aeruginosa*. Thus, these systems can actively export chloramphenicol, tetracycline and fluoroquinolones. In addition to these substrates, MexAB-OprM export B-lactams and novobiocin whereas MexXY system exports aminoglycosides (**Table 1**) [77].

Efflux pump	Pump type	Regulator	Regulator family	Inducible signal
Acinetobacter baumannii				
AdeABC	RND	AdeRS	TCS	~
Pseudomonas aeruginosa				
MexXY	RND	MexZ	TetR	Tetracycline, erythromycin, gentamicin
MexAB	RND	MexR	MarR	Superoxide stress
		NalD	TetR	~
MexCD	RND	NfxB	LacI/GalR	Biocide chlorhexidine
MexEF	RND	MetT	LysR	Chloramphenicol, GSNO
Salmonella Typhimurium				
AcrAB	RND	MarA	AraC	~
		RamA	AraC	Indole, bile salts
		RamR	TetR	~
		SoxS	AraC	~
		AcrR	TetR	~
AcrD	RND	BaeSR	TCS	Indole, zinc, copper
		CpxAR	TCS	Indole, zinc, copper
AcrEF	RND	AcrS	TetR	~
MdtABC	RND	BaeSR	TCS	Indole, zinc, copper
		CpxAR	TCS	Indole, zinc, copper
MacAB	ABC	PhoQP	TCS	Magnesium
MdsABC	RND	GolS	MerR	Gold
Staphylococcus aureus				
MepA	MFS	QacR	TetR	Rhodamine 6G, TPP
QacA	MATE	MepR		Chlorhexidine, cetrimide, dequalinium

Adapted from [73].
~ means unknown.

Table 1.
Selected multidrug resistance efflux pump regulators.

4.3 Two-component systems

Two component systems (TCS) are commonly found in bacteria, allowing them to respond to various fluctuations in the environment. Canonically, TCS are composed of a response regulator (RR) and a histidine kinase (HK) [78]. The membrane associated HKs can detect and transform various environmental sensations by autophosphorylation. The HKs can then transphosphorylate their cognate partners, the RRs, which then influence the expression of downstream genes to affect the concerned phenotype [78].

A thorough investigation of the correlation between efflux pumps and TCSs in *E. coli*, revealed the involvement of several RRs in drug resistance [79]. Among them, *mdtABC* and *acrD* expression was triggered by the BaeSR and CpxAR TCS in response to indole [80, 81] and envelope stress [79], respectively, while no signals were detected when the EvgSA TCS triggered the activation of EmrKY and MdtEF [82, 83]. Moreover, the expression of the MdtEF efflux pump was

Bacteria	Inhibitors	TCS	Mechanisms	Reference
Salmonella enterica Typhi and/or Typhimurium	XR770	BaeSR OmpR/ EnvZ	Inhibition of key interacting residues of DHp domain of HK	[89]
	NSC9608 (8 compounds, NCI library)	PhoP-Q	Inhibition of formation of the PhoP-DNA complex	[90]
Pseudomonas aeruginosa	Thiazole derivatives	Algr1-2	Inhibition of phosphorylation/ dephosphorylation of Algr2 Inhibition of DNA-binding activity of Algr1	[90]
Enterococcus faecium	Thiazole derivatives	VanR-S	Inhibition of autophosphorylation	[90]
Staphylococcus aureus	Walkmycin B and Waldiomycin	WalK-R	Binds to the HK cytoplasmic domain for the inhibition of autophosphorylation	[90]
	Salicylanilide	KinA/ Spo0F	Affects membrane fluidity, disturbing signal transduction	[90]
Methicillin-resistant Staphylococcus aureus	Bis-phenol	VanR-S	—	[90]

Table 2.
Representative TCS targets with their known inhibitors.

triggered by ArcAB-TCS system in the M9 glucose medium [84, 85]. Similar to *E. coli*, MdtABC and AcrD are also stimulated by the *Salmonella* BaeSR TCS in response to metal ions [86].

Again, PhoPQ TCS, the core virulence regulator in *Salmonella*, controls the activation of the RND type MacAB pump [87, 88]. TCS was also revealed to be involved in regulating efflux pumps in other species. In *A. baumannii*, the expression of RND type efflux pump AdeABC has been reported to be regulated by AdeRS-TCS (**Table 2**). The AdeRS-TCS regulatory system is encoded by *adeRS* genes, being positioned in the upstream region of *adeABC* genes [91]. Inactivation of AdeR or AdeS resulted in *A. baumannii* being susceptible to aminoglycosides which are the substrates of this pump, indicating the vital role of AdeRS in *adeABC* activity. The nature of these inducing signals and the AdeRS activation mechanism, however, remain unclear [92].

TCS can play an important role in drug discovery. There are several ways to target TCS proteins. Of these, the structure-based virtual screening (SBVS) analysis is carried out using compound databases containing a broad range of prospective inhibitors, including structures known to be antibacterial [93].

4.4 Biofilms

Biofilms, in both single and multi-species groups, communicate and cooperate to perform complex processes with each other and their environment [94]. With the scientists aiming to understand the intercellular interactions that encourage the development of biofilms, they are presently a serious health issue, playing a major role in abiotic device-related diseases such as catheters, prosthetic valves and contact lenses [95].

Biofilm formation can be explored in different stages comprising (a) distinctive adhesion of the planktonic bacteria (PB) to a solid surface [96], (b) micro-colonies

(MC) formation surrounded by protective secreted molecules known as the matrix of extra polymeric substances (EPS) having up to 97% water as the main component [97] and (c) dispersal including shedding of PB or MC from the mature biofilm [97]. The last phase can encourage further biofilm colonization of the host which can eventually benefit the bacteria with a limited supply of nutrients and waste accumulation [97]. Importantly, the transition from planktonic growth to surface life is triggered by several environmental signals known as various stresses for the bacteria based on their ecological niche [98]. These include UV radiation, pH changes, oxygen tension, osmolarity, iron availability, temperature, nutrient supply and desiccation [98], which may disrupt their fundamental functions such as growth and survival capability. The environmental indications, however, vary significantly between organisms. Thus, *P. aeruginosa* will form biofilms under most circumstances [99, 100] while *E. coli* O157:H7 produce a biofilm under low-nutrient conditions only [101].

Recent advances in biofilm research have proven its connection to various pathways and proteins [61]. For instance, defects in MDR EP activity reduced the biofilm formation and thus, EP inhibitors have been employed as a promising biofilm inhibition approach for strains of *E. coli* and *Klebsiella* [102], *Salmonella* [103], *P. aeruginosa* and *S. aureus* [104]. However, certain other reports show that despite the elimination of planktonic cells through pharmacological intervention, the sessile forms are resistant and continue to proliferate within the biofilm [105]. This is more of prominence on abiotic surfaces [95], such as catheters [106], contact lenses [107] and prosthetic cardiac valves [108]. Thus, alginate mucoids, with EPS overexpression, from *P. aeruginosa* species isolated from cystic fibrosis patients, were found to improve AMR by promoting the biofilm formation [109].

5. Conclusion

The constant increase in AMR is a significant public health concern that needs to be addressed now. This review starts with an introduction to AMR followed by the threats from the clinically important MDR pathogens and their rise. With the existing management strategies for MDR by the scientists still ongoing, we have taken up this study to propose an integrated approach to deal with MDR threats. Thus, the review ends with new connections of important bacterial components with MDR.

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Conflict of interest

The authors declare no conflict of interest.

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