



Strategies to combat antimicrobial resistance in Indian scenario

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Received: 3 July 2021; Accepted: 12 July 2021

ABSTRACT

Antimicrobial resistance (AMR) is one of the major public health crisis recognised globally. Microbial infections cause significant productivity losses in animals and humans. In livestock, these microbial infections reduce the growth rates and fertility, diminish production of meat and milk, and occasionally lead to mortality, and are therefore, a major concern for animal welfare. In the dearth of alternative prophylactic measures, antibiotics remain the principal tool for their management. Once an antibiotic is used rampantly, resistance against it is inevitably seen in the microbe population and the hunt for a new drug grows. Discovery and development of a new antimicrobial drug is a time taking and expensive procedure with limited assurance of success. As a result, the past few decades have witnessed only a very few new classes of antibiotics. If the AMR can be restricted or reverted, the success rate of antimicrobial therapy can be boosted and many public health issues be avoided. All these ask for a comprehensive plan to prevent or reduce the antimicrobial resistance and economic losses to the animal husbandry sector. The present review provides an overview of AMR in India, mechanism of its occurrence and the possible roadmap to combat the emerging threat of AMR in Indian scenario.

Keywords: Antimicrobial resistance, Combination therapy, Immunomodulation, Interventions, Mechanism

The story of antibiotics started way back in 1928 by the discovery of penicillin followed by prontosil, chloramphenicol, aminoglycosides, tetracycline and so on. Later on, their analogs were initiated for taking care of the drawbacks leading to breakthroughs in treatment of bacterial disease. In 2019, WHO recognised 32 antibiotics in clinical development, of which only 6 antibiotics were classified as 'Ingenious'. Initially, the word 'antibiotic' referred to a substance produced by one living organism that selectively inhibited the growth of another microorganism. Later on synthetic molecules took over the major share. Ever since antibiotics were added to the armamentarium to manage notorious bacteria and the bacteria started looking for alternate mechanisms to evade the killing and now its success has assumed a giant figure in the form of antimicrobial resistance (AMR).

Today, AMR is recognized as one of the most critical public health challenges of the 21st century. According to Epidemiological surveillance networks in Europe and Asia European [Antimicrobial Resistance Surveillance Network – EARS - Net, Central Asia and Eastern European Surveillance of Antimicrobial Resistance - (CAESAR)] antibiotic resistance has now become much more extensive

during last decades (Migliori *et al.* 2018, WHO 2017). In the USA, the expected number of people suffering with antibiotic-resistant infections is more than 2.8 million each year. Moreover, the casualties are more than 35,000 with hospitalization of nearly 223,900 people. Worldwide approximately 700,000 deaths can be characterised to microbial resistance and by 2050 it is estimated that the death toll may rise up to 10 million annually (O'Neill 2014). The epidemiological risk and hazards to global health security posed by AMR has been reiterated in several World Health Assembly (WHA) declarations. AMR has been prioritized under the Global Health Security Agenda (GHS), and India is one of the contributing countries. The Indian Ministry of Health and Family Welfare (MoHFW) has also identified AMR as one of the top priorities for collaborative work with WHO.

India ranked fifth in antibiotic consumption in food producing animals (ruminants, pigs, and poultry) in 2010, and with mounting incomes and shifting in dietary patterns in favour of the consumption of animal protein, especially for poultry, the veterinary antibiotic utilization is projected to further rise by 312%, elevating India to the fourth rank by 2030 (Van Boeckel *et al.* 2015). India has already reported some of the peak resistance rates among common commensal and environmental bacteria against antibiotics. To quantify the burden of AMR in food-producing animals and aquaculture in India, ICAR has joined hands with FAO and has initiated a Network project (Indian Network for Fisheries and Animal Antimicrobial Resistance, INFAR),

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with a mandate to identify strategies for prevention and reduction in the emergence and spread of AMR in aquaculture and food animals. It is presently functioning in 20 centres (9-fisheries and 11-livestock sector) across the country, covering animal, foods of animal origin, aquaculture, environment and human hospital settings. In its third annual meet in September 2020, in the Fisheries component, it has reported the highest incidence of AMR against ampicillin (36%), followed by enrofloxacin (30%) amoxicillin/clavulanic acid (25%) and cefotaxime (25%) in *E. coli* strains while *Vibrio parahaemolyticus* isolated from shrimps and marine fish was mainly resistant to cefotaxime (54%), ampicillin (47%) and ceftiofur (43.9%). Resistance to ceftiofur was comparatively higher (31%) in CONS as compared to *S. aureus* isolates (8.5%). The livestock sector emphasized on the epidemiologically importance of *S. aureus* and *E. coli* in Indian context in a one-health environment with high prevalence of methicillin resistance in the dairy sector in Karnataka and Assam. Significant level of multi drug resistance in *S. aureus* has also been reported in humans and animals in Uttar Pradesh based on antibiotic sensitivity assay and presence of *mecA* genes (Kutar *et al.* 2015, Sharma *et al.* 2015, Jayshree *et al.* 2016, Yadav *et al.* 2018a,b). To further add to this, resistance to the broad-spectrum fluoroquinolones and third generation cephalosporin antibiotics is already more than 70% in *Escherichia coli*, *Klebsiella pneumoniae*, and, *Acinetobacter baumannii* and more than 50% in *Pseudomonas aeruginosa*. Even the microbial resistance to carbapenems, the last-resort antibiotics, is extremely high among gram-negative microorganisms (Anita *et al.* 2014, Gandra *et al.* 2016).

What is antimicrobial resistance (AMR)?

AMR refers to the decrease in sensitivity of the microorganism to the antibiotic, clinically evident either as an increase in MIC (minimum inhibitory concentration) or total insensitivity. AMR naturally occurs over time, generally due to genetic or phenotypic changes. Genetic AMR occurs when the bacterial population grows in the presence of highest concentration of antibiotics up to its four fold, i.e. higher than MIC while MIC is minimum inhibitory concentration is the lowest concentration of antibiotics that habitually prevents visible growth in permissive growth culture medium of same bacterial species. On the other hand, phenotypic AMR results when bacterium change, arise and subside with a particular cell without dependence on genetic change in the same population, i.e. relative to sensitive cells (O'Neill 2014, Rajput *et al.* 2018, CDC 2019). Phenotypically, AMR can be manifested as a gradual decline in the sensitivity towards the antibiotic or it can be a complete insensitivity. In the former case, the higher concentrations of the antibiotic continue to treat the infection but in the latter case, a complete failure of treatment is observed. Though the second type usually involves a gene transfer, it may also be due to a single mutation as in case of fluoroquinolone

resistance in *Campylobacter jejuni* (Gootz and Martin 1991).

AMR can be constitutive or induced. Constitutive resistance is observed when the bacteria is naturally deficient in the target of the antibiotic as seen in case of Chlamydia for β -lactams owing to the absence of peptidoglycan in the cell wall. It can also occur naturally over a period of time. Genes encoding resistance may be present in the bacteria like antibiotic producing one to protect themselves or it can arise due to change in chromosome by the sudden impulsive mutations. Recent database enlists more than 20,000 potential resistance coding genes of nearly 400 diverse types, envisaged chiefly from existing bacterial genome sequences (Liu and Pop 2009). Fortunately, practical resistance determinant count in pathogens is much smaller (Davies and Davies 2010).

Major concerns of antimicrobial resistance

The increasing AMR incidences globally can be attributed to certain factors that are critically responsible for the spread of AMR (Fig.1). These include the lack of awareness regarding the use of antibiotics and their after effects in dairy farmers, poultry breeders and other stakeholders. It generates the major factor related to antibiotics-induced AMR improper drug and dose regimen that includes the dose, dosing interval, duration of treatment and the formulation. Use of too high or too low dose, over prescription of drugs, and incorrect duration of prescription usually intensifies the problem (Ayukekbong *et al.* 2017, Bello-López *et al.* 2019). Lack of awareness further leads to the indiscriminate use of antibiotics for treatment of diseases and as growth promoters. Indiscriminate use of antimicrobial is usually the primary driving force for AMR in animal husbandry, removing the sensitive population and allowing proliferation of the mutant strains. Even the

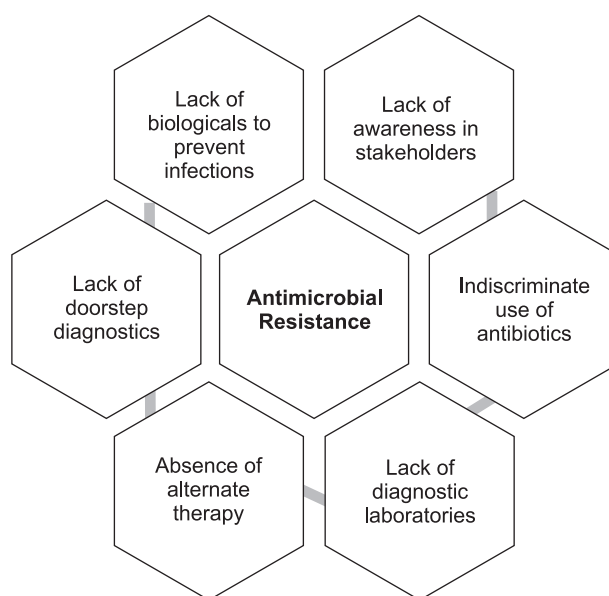


Fig. 1. Major concerns of Antimicrobial Resistance (AMR)

commensal bacteria like *E. coli* and Enterococci can serve as a reservoir for such resistance genes and can transfer them as and when conditions are favourable and are therefore, used internationally as indicators for prospects of Gram-positive and negative bacterial resistance, respectively (Ayukeybong *et al.* 2017). It is the genesis of three critical conditions that determine the spread of AMR presence of a resistant mutant in the bacterial population; chances of vertical or horizontal transmission of resistance genes; selection pressure of antibiotics (Drlica 2007). In Indian scenario, lack of facilities to detect drugs of choice and perform antibiotic sensitivity to support the selection of effective drugs further contribute to AMR spread as it has been reported in food borne pathogens, wild birds, domestic animals and humans (Kumar *et al.* 2010, Kumar *et al.* 2011, Kumar *et al.* 2013a,b, Malik *et al.* 2013, Suman *et al.* 2020).

When the drug is administered repeatedly, the plasma or bioavailable concentration of the drug rises after each administration and degrades according to the principles of pharmacokinetics. As the total bacterial population comprises of different subpopulation, a rise in the drug concentration will definitely increase the population of bacteria killed, and above a certain level, MPC (Mutant Prevention Concentration), all bacteria will be inhibited or killed (Drlica and Zhao 2007). As the concentration falls below MPC, the native bacteria will still be killed but the resistant mutants will be favoured for survival till the concentrations further fall to selective concentration, i.e. the concentration at which not even the susceptible bacteria will be killed. In other words, the selection pressure will work between the mutant prevention concentration and the selection concentration, i.e. the selection window (Drlica and Zhao 2007). The cost of treatment with antibiotics further aggravates the situation. To prevent AMR, a smaller selection window is advisable which is again under the influence of dose and treatment interval. High loading dose along with regular treatment interval can reduce the selection window to be minimal (Ayukeybong *et al.* 2017). The use of indigenous traditional knowledge (ITK) based treatment can be an alternate therapy. It requires the development and validation for cost effective, safe and easily available alternate medicines. It can reduce the presence of resistance genes in existing microbial population as chromosomal genes are transferred vertically while genes on plasmids, transposons, integrons, etc. are spread horizontally and more efficiently and receive further favour from resistance selection pressure induced by the intensive use of antimicrobials (Ayukeybong *et al.* 2017, Bello-López *et al.* 2019) as observed in zoonotic *Salmonella* Typhimurium. Once resistance develops, the mutated gene is transferred to next generation during replication. In the presence of antibiotic in the environment, wild type is killed and the bacteria with resistance genes are allowed to flourish. The bacterial survival is influenced by the multiple factors like effect of resistance genes on bacterial fitness and selective pressure imposed by the rate and pattern of

antibiotic. Approximately 85% of therapeutic failure in β -lactam, ampicillin, sulfonamide/trimethoprim, aminoglycosides and peptide like vancomycin is due to conjugation (Davies and Davies 2010, Ayukeybong *et al.* 2017), perhaps utilizing plasmids, transposons, or integrons. These incidents can be reduced by adopting the policy 'prevention is always better than cure'. The availability of safe, effective and user-friendly biological vaccines against infectious diseases can avoid infections.

All these concerns can be addressed by the development of a national action plan on antimicrobial resistance to optimize the use of antimicrobials through public awareness, the behaviour change of all the stakeholders regarding the approach of treatment and use of antibacterials in feed as growth promoters. It is possible only by setting up a two way communication with authorities and stakeholders. The action plan should also focus on innovation in the treatment through research and development of newer drugs including ITK based alternate medicine. The access to these drugs and therapies to all is another key factor. Simultaneously continuous surveillance and monitoring is required for the need based changes in strategies and planning. These can be implemented by the development of Sustainable Development Goals (SDGs) (Fig. 2).

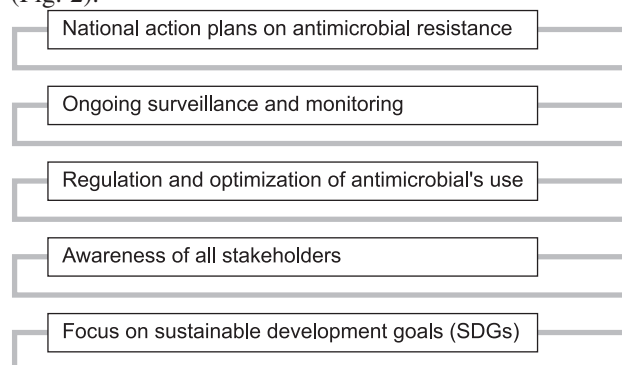


Fig. 2. Major focus areas to combat antimicrobial resistance (AMR).

Mechanism of antimicrobial resistance

The main mechanisms causing AMR include chemical modification of antibiotics, systemic elimination of antibiotics by efflux pump, modification of drug target and alternate defense strategies of pathogenic bacteria like biofilm formation (Rajput *et al.* 2018) (Fig. 3). The entry of antibiotics into the bacterial cell can be avoided by altering permeability. *P. aeruginosa* develops resistance to imipenem by loss of porin proteins owing to mutation, thereby altering the outer membrane permeability of the bacteria (Lister *et al.* 2009). In addition to this, there are 5 families of efflux systems in bacteria which disallow the antibiotics to find their intracellular targets by exporting the antibiotics as observed in case of macrolide resistance in *Streptococcus pyogenes* where it is encoded by *mefA* gene in the bacteria and is specific for 14- and 15-membered macrolides (Kaplan 2003, Kourtesi *et al.* 2013).

Bacteria may also produce enzymes that chemically

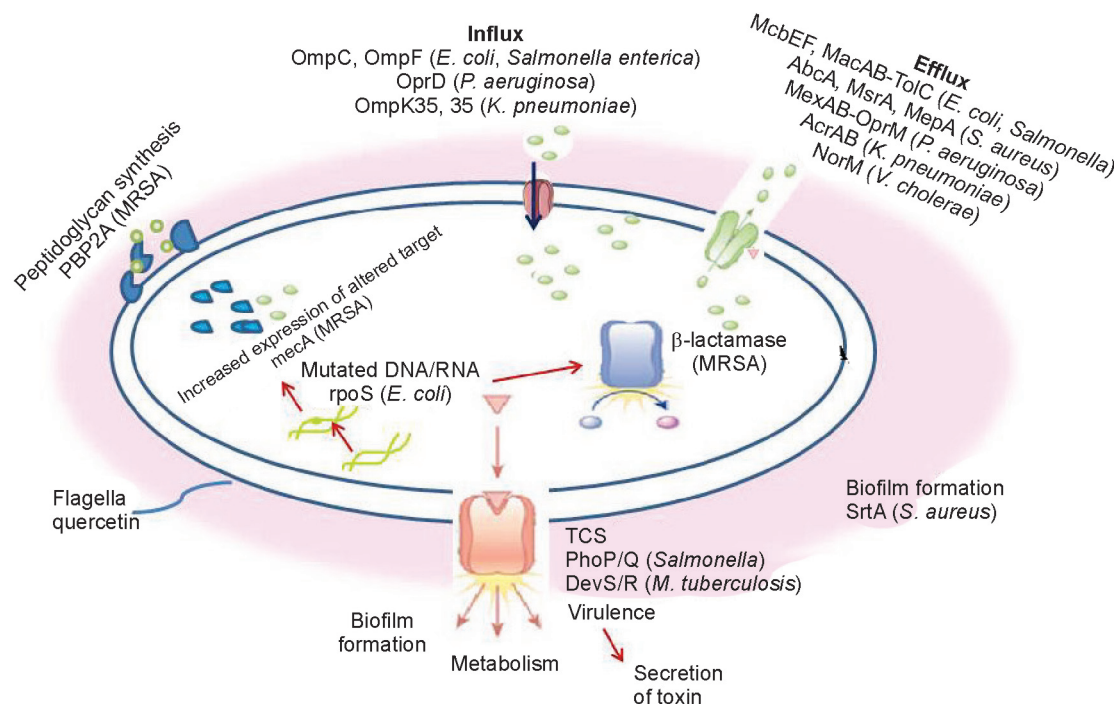


Fig. 3. Mechanism of antimicrobial resistance in bacteria.

breakdown or degrade antibiotic molecules. The most primitive example is production of enzyme β -lactamase in *S. aureus* that inactivates the penicillin by hydrolyzing the β -lactam ring. On the other hand, the normal drug's targets may get altered or replaced in bacterial cells like MRSA (Methicillin resistance in *S. aureus*) due to the presence of *mecA* gene. These genes are encoded for PBP2A that confers the resistance against all β -lactam antibiotics, cephalosporins and carbapenems together with β -lactamase inhibitor combinations (Levy 1998). The PBP2A has lower affinity for β -lactams as compared to PBP4. Other than these, production of biofilms is a major virulence factor as it shields the bacteria from antibiotic exposure as well as phagocytosis, and promotes overall persistence of the microorganisms (Hoiby *et al.* 2010). Active efflux can function synergistically with other mechanisms of resistance, for instance, in *E. coli* strain expressing both beta-lactamases and efflux pumps, and which is insensitive to beta-lactams (Zhan *et al.* 2004) and quinolones (Davin-Regli *et al.* 2008).

Modalities to cure antimicrobial resistance

AMR is a complex problem. India is a hotspot for antimicrobial use and an adequate knowledge of factors contributing to their amplified use only can guide the designing of AMR intervention strategies. Attempts to improve inappropriate antibiotic usage, wherever possible, should be the first priority.

Pharmacokinetic consideration: All the drug molecules enter the site of action through passive diffusion but, being a xenobiotic, they are actively extruded by the cellular defense procedures. For therapeutic success, a minimal

therapeutic level of drug needs to be maintained for a certain duration of time. By hypothesis, the antibiotic drug therapy can be made effective if the activity of efflux pumps in the bacteria is reduced or their expression is masked, or else the drugs are redesigned, so that they are no longer suitable substrates for the efflux systems, and thus their clinical efficacy is restored (Kourtesi 2013). Out of the three rational approaches in the direction of confronting drug resistance, reducing the efflux of clinically relevant antibiotics, probably through a potent efflux pump inhibitor or a modulator seems to carry a great prospective.

Efflux pump modulation: All the major efflux pump families- MFS, ABC, SMR, and MATE are widely distributed in Gram-positive and Gram-negative bacteria, while the RND superfamily is specific to Gram-negative microorganisms. Efflux pump activity modulators have been clinically proven to either decrease the intrinsic bacterial resistance to antibiotics or quash the resistance acquired in resistant strains, even with multiple target mutations, and also diminish the frequency of emergence of new resistant (mutant) strains (Lomovskaya *et al.* 1999). Over expression of efflux pumps in clinically resistant strains has already been observed (DeMarco 2007). The over expression of efflux pumps can be induced by the antibiotics itself during the course of treatment or by unrelated drug molecules and host-produced endogenous molecules (Piddock 2006), accounting for the slow development of refractoriness in the course of chronic treatment. *S. aureus* exposed to increasing concentrations of ethidium bromide develop higher levels of resistance to fluoroquinolones and biocides compared to the parent strain, and this increase in resistance was related to a several-fold

increase in the expression of *norA* efflux gene, which in turn was due to a deletion in its promoter region (70 bp). Interestingly, a single modulator can affect the activity of multiple efflux pumps. Paroxetine, a serotonin reuptake inhibitor, inhibits drug efflux by NorA as well as other non-MFS drug efflux pumps, such as those of the MATE family (Kaatz *et al.* 2003, Kuroda and Tsuchiya 2009). Celecoxib alone is not bactericidal but in combination at lower concentrations increases the sensitivity of the bacteria to the antibiotics, probably due to the inhibition of efflux transporters that are involved in extruding antibiotics out of bacterial cells (Kalle and Rizvi 2011).

Several natural molecules eg. plant alkaloid reserpine, kaempferol rhamnoside, and capsaicin inhibit activity of efflux pump NorA of *S. aureus* (Holler *et al.* 2012). Another MFS multidrug efflux pump, *bmr* is closely related to NorA from *S. aureus* (Neyfakh, 1992). Reserpine modulates the activities of two dissimilar MFS-associated chloramphenicol efflux pumps, CmlR1 and CmlR2, from Gram-positive *Streptomyces coelicolor* (Vecchione *et al.* 2009). Reserpine could also inhibit the drug efflux pump, Lde, which confers resistance to the fluoroquinolones ciprofloxacin and norfloxacin present in Gram-positive pathogen *Listeria monocytogenes* (Godreuil *et al.* 2003).

P-glycoprotein (P-gp), one of the key members of the superfamily of the ATP-binding cassette (ABC) efflux proteins, recognizes many antibiotics as its substrate (Van Bambeke *et al.* 2000, 2003), and could therefore effectively modulate their intracellular concentration and hence their activity. P-gp-mediated efflux of azithromycin partially reduced its cellular accumulation and hence, diminished the antimicrobial activity towards phagocytized *S. aureus* (Seral *et al.* 2003a, b).

Pharmacodynamic consideration: In contemporary years, discovery of microbial quorum sensing (QS) has raised new hope for studying the regulatory mechanism of drug resistance. Bacteria adapt to changes in their environment through quorum sensing (QS) using a two-component regulatory system (TCS). It involves at least two proteins, namely the sensor kinase (to sense external stimuli including exposure to antibiotics and biochemical alteration in the local milieu) and the response regulator (decides the expression profile of bacterial genes for survival and adaptation). Sensing antibiotics in the immediate environment results in activation of bacterial defences and alterations in cell physiology that augment antibiotic resistance. This system is ubiquitous in bacteria, but absent in mammalian cells, so tapping it may reduce the burden of bacterial virulence and drug resistance/tolerance as well, which makes it an attractive target for multi-drug resistant antimicrobial therapy (Zhao *et al.* 2020).

QS depends on the synthesis, release, and uptake of autoinducers (AIs) in the local milieu. These signaling molecules include *N*-acyl homoserine lactones (AHLs) produced by LuxI family of proteins of Gram-negative bacteria, oligopeptides (autoinducing peptides, 5–34 amino

acids residues) in Gram-positive bacteria, and AI-2 employed by both Gram-positive and Gram-negative bacteria for intercellular communication. These extracellular signaling molecules accumulate in the environment in proportion to cell density (Kabir *et al.* 2010, Deep *et al.* 2011, Krishnan *et al.* 2012) and regulate a number of bacterial physiological processes, together with virulence, motility, luminescence, biofilm formation, sporulation, development of genetic competence, synthesis of peptide antibiotics, production of secreted proteolytic enzymes, and fluorescence (Singh *et al.* 2009, Rocha-Estrada *et al.* 2010). In Gram-positive bacteria, the precursor peptide AIs are effluxed out by ATP-binding cassette complex and once the concentration of the peptide AIs reaches the threshold value, the sensor kinase protein gets activated and phosphorylates the response regulator, which then binds to the target promoter and leads to QS gene regulation (Xavier and Bassler 2003). On the other hand, in case of Gram-negative bacteria, the AIs diffuse out freely to reach the threshold value, and generate a positive feedback loop to synthesize more AIs (Zhao *et al.* 2020).

TCSs are major players in the realm of infectious disease caused by pathogenic bacteria. QS shares a critical role in the pathogenesis of *Salmonella*, for instance epithelial cell invasion and intramacrophageal survival through PhoP/PhoQ (Fields *et al.* 1989, Miller *et al.* 1989). Radicicol, an ATP competitor, inhibits the auto-kinase activity of PhoQ (Guarnieri *et al.* 2008). PhoQ also activates another response regulator PmrA via a small connector protein, and ultimately contributes to induction of resistance against cationic antimicrobial peptides, like polymyxin B (Kato and Groisman, 2004, Olaitan *et al.* 2014). Polymyxins are often employed as an alternative 'last-resort' drug in the treatment of Gram-negative bacterial infections that are resistant to conventional antibiotics, such as aminoglycosides, quinolones, and β -lactams, including carbapenems (Zavascki *et al.* 2007). PhoP/PhoQ system is also vital for the virulence of *Shigella species* (Moss *et al.* 2000). Therefore, the inhibitor of PhoQ could be a potential supportive drug in polymyxin B therapy. Similarly, DevS/DevR of *M. tuberculosis* is required for survival under hypoxic conditions associated with non-replicating dormant periods within host cells. Peptides analogs of the N-terminal domain of DevR can reduce bacterial survival in anaerobic cultures (Kaur *et al.* 2014).

Bacterial biofilms protect the microbes from antimicrobial agents as well as phagocytosis. Biofilm is a matrix of bacteria's self-produced extracellular polymeric substance in which a complex aggregate of cells are embedded. Several phytochemicals restrict biofilm formation in major pathogens. Berberine, in modest concentrations, significantly inhibits biofilm formation in *S. epidermidis* (Wang *et al.* 2009). Quercetin produces noteworthy reduction in QS-dependent violacein production, biofilm formation, motility, and alginate production in a concentration-dependent manner. It also

acts as a competitive inhibitor for signaling compounds toward the lasR receptor pathway (Gpou *et al.* 2015) and hinders protease, pyocyanin, and elastase synthesis at a lower concentration (Ouyang *et al.* 2017). Trans-cinnamaldehyde, from cinnamon bark (Amalaradjou *et al.* 2010, 2011), terpenes such as carvacrol, thymol and geraniol from citrus peel, essential oils of *Cymbopogon citratus* and *Syzygium aromaticum* exhibit marked antibiofilm activity against both fungal (Dalleau *et al.* 2008, Khan and Ahmad 2012a,b) and bacterial biofilms (Nostro *et al.* 2007, 2009, Knowles *et al.* 2005). Lemongrass oil inhibits production as well as can destroy the pre-formed biofilms (Moore-Neibel *et al.* 2012).

Traditional approaches to overcome AMR

Vaccination: Vaccines can curb AMR both directly and indirectly. Vaccination reduces the emergence of a resistant strain. Secondly, vaccines are prophylactic drugs which reduce the spread of pathogens, which substantially reduce the frequency of clinical prescriptions and diminish the chance of circulation of resistant strains. Livestock sector accounts for more than half of global antibiotic use (Oliver *et al.* 2011) and is an important driver of AMR (McEwen and Fedorka-Cray 2002). Use of vaccines in food-producing animals can substantially decrease antibiotic use and reduce the zoonotic risk of the emergence of antibiotic resistance (Hoelzer *et al.* 2018).

Immunomodulation: Impaired host immunity is a threat for AMR. Immunomodulation of the host can lead to an increased disease resistance and indirectly reduce AMR. Activation of macrophage is a primary requisite for innate immunity. Innate memory of the immune system is being strategized to produce modulated response to disease stimuli by producing epigenetic alterations in chromatin and metabolic reprogramming of innate immune cells. Vaccines are effective immunomodulating agents, which prime the host immune system and also maintain a memory. Phytochemicals such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids and glycosides, are also worth mentioning immunomodulatory agents (Jayati *et al.* 2013). They can reduce the infectious pathophysiology and selection pressure leading to alteration in antibiotic demand as well as alter pharmacokinetic profile of the antibiotic in clinical cases through multiple pathways. Oil from seeds of *Chenopodium ambrosioides* and Eucalyptus oil (Cruz *et al.* 2007) enhance phagocytosis, whereas essential oils from *Petroselinum crispum* (Yousofi *et al.* 2012) and *Artemisia iwayomogi* (Ryul *et al.* 2003) can suppress phagocytosis. In addition to phagocytosis, other markers of intracellular pathogens killing like, increase in production of NO and ROS alongwith cytokine secretion can also be stimulated. Wagonin, a flavonoid of *Scutellaria baicalensis* causes increased production of NO (Jen *et al.* 2002) while *Embllica officinalis* enhances ROS generation (Suja *et al.* 2009). Enhanced macrophageal secretion of IL1, a key inflammasome and immunological mediator, with extracts of *Aloe vera* (Pugh *et al.* 2001, Nascimento *et al.* 2006),

Astragalus radix (Song *et al.* 2000), *Ganoderma lucidum* (Wang *et al.* 2002) and increase in TNF α secretion in the presence of ursolic and oleanolic acids and *Ziziphus jujube* extracts (Lopez-García *et al.* 2015) are already well documented. Many plants extracts have been recommended for their effective antibacterial activities (Kumar *et al.* 2011, Vashney *et al.* 2012, Sharma *et al.* 2014, 2019). Moreover, these have been tested against different bacterial pathogens including *E. coli*, *Staphylococcus* spp., *Pseudomonas* spp., *Kelbsiella* spp., *Campylobacter* spp., *Streptococcus* spp. and many more (Kumar *et al.* 2010, Kumar *et al.* 2011, Kumar *et al.* 2012, Kumar *et al.* 2013, Verma *et al.* 2014, Upadhyay *et al.* 2020).

Replacing antibiotics in feed with prebiotics, probiotics and synbiotics: Antibiotics are used as animal feed premix in small (sub-therapeutic) doses to promote growth, and contribute to development of AMR. The use of antibiotics as growth promoters has been forbidden in many countries including the European Union but have regulated use in India. *Prebiotics* are nondigestible food supplements that selectively stimulate the growth and/or activity of beneficial bacteria while *probiotics* are supposed to promote beneficial bacteria over pathogenic populations. Synbiotic contain both prebiotic and probiotic effects. Synbiotics can replace antibiotics in animal feed for enhancing the immune function, growth performance and productivity in animal rearing but safety concerns in probiotic strains remain on acquired resistance genes that could be transferred via conjugative plasmids, transposases, and bacteriophage elements.

Diarrhea is usually accomplished owing to abnormal bacterial colonization. Probiotic bacteria including *Bifidobacteria*, *Lactobacillus*, *Bacteroides*, *Escherichia coli*, *Saccharomyces cerevisiae* var. *boulardii* and *Bacillus coagulans* may exert their beneficial effects by restoring or supplying the essential commensal strains necessary for protection against intestinal inflammation and injury. Prebiotics and postbiotics, individually, may also accomplish this goal, through the promotion of commensal growth or by imitating commensal activity, respectively. In addition to promoting the growth of beneficial commensals, prebiotic therapy may also improve intestinal motility and gastric emptying, resulting in improved feeding tolerance (Indrio *et al.* 2009a, b) and better gut immunity, thus, reducing the need of antibiotics in diarrhea cases (Patel and Denning 2013).

Newer approaches to overcome AMR

Combination therapy: Although AMR typically involves modification of the drug or its target and/or decrease in bioavailable concentration of drug either by decreased influx and/or an increased efflux, the bacterial response to antibiotics can be affected by chemicals or secondary metabolites of plant, without their being bactericidal, in several ways, thereby affecting the bacterial survival. They can inflate the antimicrobial spectrum of antibiotics as well. These may include anatomical and/or physiological

disruption of membrane (including the efflux) (Sanchez *et al.* 2010, Chitemerere and Mukanganyama 2014), interruption of transcription or translational processes (Anandhi *et al.* 2014), intervention with intermediary metabolism, induction of coagulation of cytoplasmic constituents and interruption of quorum sensing (Nazzaro *et al.* 2013, Radulovic *et al.* 2013, Mogosanu *et al.* 2015). Imidazopyridineaminofurazan (IPA) an inhibitor to bacterial Penicillin-binding protein and Serine/Threonine kinase-Associated (PASTA) kinases is beneficial in regulating resistance to β -lactam antibiotics (Schaenzer *et al.* 2017). Omega 3 and oleic acids get incorporated to the outer cell membrane of Gram negative bacteria to increase its permeability. Extracts of *Berberis aristata* and *Camellia sinensis* inhibit hemolysin and hemagglutination on the bacterial membrane (Thakur *et al.* 2016). *Aegle marmelos* prevent binding of bacterial toxin and colonization of the intestinal epithelial cells to treat bacterial diarrhea (Brijesh *et al.* 2009). MIC of tetracycline and erythromycin reduces synergistically in presence of ethanolic extract of *Mangifera indica* (Souto *et al.* 2011) while that of tetracycline is halved against *Salmonella* Typhimurium and one-fourth against *K. pneumoniae* when combined with methanolic extract of *Tecton grandis* (Purushotham *et al.* 2010).

Berberine shows synergism with β -lactam against MRSA (Yu *et al.* 2005), perhaps by potentiating antimicrobial action by blocking NorA over expression (Stermitz *et al.* 2000). In case of bactericidal phytoactive compounds, even sub-lethal concentrations can influence virulence in Gram-positive (Upadhyay *et al.* 2013, Mooyottu *et al.* 2014) and Gram negative bacteria (Upadhyay *et al.* 2013) and fungal pathogens (Yin *et al.* 2015) by modifying transcription of DNA/RNA (Goh *et al.* 2002, Qiu *et al.* 2010), translation

of proteins (Qiu *et al.* 2010) and quorum sensing (Koh *et al.* 2013, Ahmad *et al.* 2015).

Antibiotic resistance breakers: Natural compounds can reverse AMR. Methicillin resistance in MRSA is primarily due to the *mecA* gene which encodes for PBP2A. Phenolic phytoconstituents like curcumin, tiliroside, pinoresinol, magnatriol B and momorcharaside B target *mecA* and PBP2A to curb AMR (Fig. 4). Apigenin, from chamomile flowers, reverses resistance of MRSA when used along with ampicillin and ceftriaxone (Akilandeswari *et al.* 2016) with a drastic decline in MIC for ampicillin (800 to 107 $\mu\text{g/mL}$), and ceftriaxone (58 to 2.6 $\mu\text{g/mL}$). Similarly, morin, a flavonol from guava leaves, also reverses oxacillin- and ampicillin resistance in MRSA (Mun *et al.* 2015) by reducing PBP2A expression. Similarly, flowers of *Duabanga graniflora* also inhibit PBP2A in presence of ampicillin (Santiago *et al.* 2015). Essential oils from *Teucrium ramosissimum* and *Pistacia lentiscus* also reduce the resistance of Penicillins' group in MRSA (Lahmar *et al.* 2017). Certain natural products eg. *Phellinus baunii* extracts also act synergistically when used in combination with otherwise resistant MRSA by targeting PBP2A (Hong *et al.* 2016).

Engineered bacteriophages: Bacteriophages are living, dynamic, evolving, and specific viruses to treat bacterial infections. They can also be an alternative or synergistic to antibiotic use (Chaudhry *et al.* 2017). This therapy was introduced in the early 1920s in Georgia and later on in whole Eastern Europe and also western countries. The specificity of phages makes it possible to target only the pathogen responsible for the infection to be treated, and thus help in preserving the commensal microbiota. Phages can be a wonderful alternative only if their coevolutionary

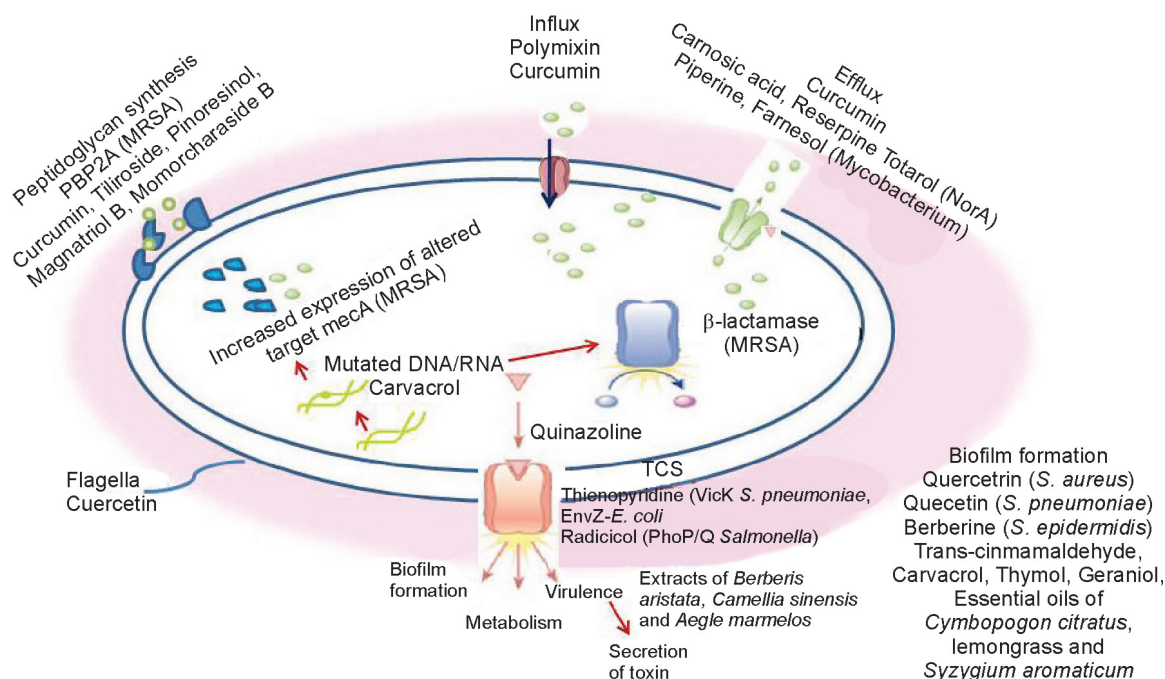


Fig. 4. Herbal interventions to combat antimicrobial resistance.

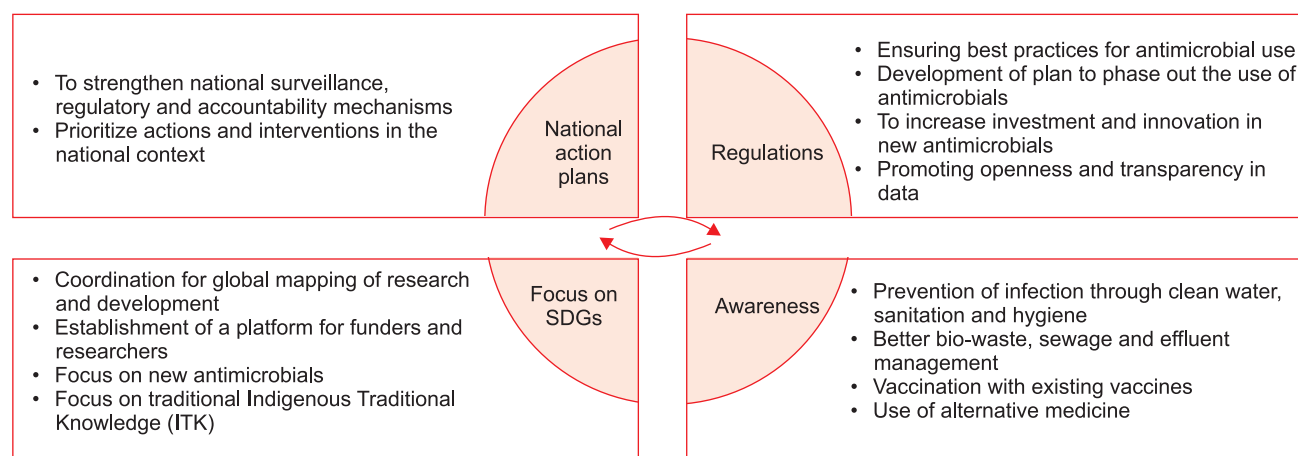


Fig. 5. Main theme areas to overcome antimicrobial resistance.

capacities can be compromised. Lumiphage, a bacteriophage-based therapy, has recently been commercialized by ICAR for shrimp hatcheries as an alternative to antibiotics to control significant mortalities of shrimp larvae due to vibriosis. It is basically a cocktail of phages that can neutralize a wide range of specific pathogenic bacteria in the hatchery settings.

Microbiome: The microbiome present on skin and other mucous membranes consists of beneficial viral, fungal, protozoans, archaea and bacteriophage flora, and is one of the body's best defense mechanisms. It plays an important role in digestion, and immunity (Bull and Plummer 2014, Ferreira *et al.* 2014). It synthesizes and releases several broad spectrum antimicrobial peptides which have recently been considered as the Achilles' Heel of antimicrobial resistance (Lewies *et al.* 2019). Maintaining a healthy biome will certainly reduce the disease incidence and the antibiotic usage. The relationships between microbiome and antibiotics can work in the opposite direction as well. Antimicrobials may alter the composition of the microbiota, inflaten the host antimicrobial-resistance gene pool, and ultimately degrade the protective effects of the microbiota against invasion by pathogens (Ferreira *et al.* 2014, Lewies *et al.* 2019).

Conclusion

To overcome AMR, understanding the mechanisms of bacterial response, specifically to disease pathophysiology and drug treatment is inevitable. Selective inhibition of pathogen efflux pumps and its defence mechanism along with herbal resistance breaker adjuncts, to target host factors and block bacterial virulence factors, can lead to better bacterial killing and will overcome the rising problem of drug resistance. Other tools such as vaccines, phages, and microbiota targeting are emerging strategies that can complement antibiotics against AMR.

There is no single solution, and a globally integrated strategy is an obligation to combat AMR effectively. AMR needs to be addressed in global scenarios that need comprehensive policy (Fig. 5) that include a

multidimensional approach with the involvement of all stakeholders in the national programme.

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