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Review Article

SUPERBUGS AND RECENT CONTROLLING APPROACHES: A MINI REVIEW

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Abstract: Antimicrobial resistance (AMR) is a growing threat to human and animal health, reducing the ability to treat bacterial infections and consequently the risk of morbidity and mortality caused by resistant bacteria. The World Health Organization (WHO) has predicted 10 million deaths by 2050 due to infectious diseases. A considerable increase in the number of pathogens harbor multidrug resistance genes, seemed to be arising from the extensive use of antimicrobial agents for medical issues particularly in food-producing animals, along with the many pathways of their release into the environment. The emergence of multidrug-resistant (MDR) microorganisms has threatened the longstanding use of antibiotics, which were globally used to save millions of lives. The evolution and spread of AMR have a great adverse impact on human beings. Naturally, bacteria have the genetic ability to acquire and spread resistance to therapeutic agents. Therefore, the major challenge has been to limit the extensive and the inappropriate overuse of antibiotics, in addition to finding new drugs either from synthetic or natural origin to counter microbial resistance by acting on the specific target responsible for MDR. To overcome the crisis of antimicrobial drug resistance, WHO has issued several guidelines on the use of antimicrobial agents, such as preserving antibiotics that are important for human health and preventing their usage in agriculture. Several therapeutic approaches like vaccines, antimicrobial peptides, bacteriophages, and nanotechnology, along with phytochemicals and herbal medicines, are applied in *vitro* and in *vivo* experiments and are supposed to be suitable candidates for antimicrobials to combat the rapidly evolving AMR. In this review, we will discuss the causes of this resistance, types, its spread between ecosystems, superresistance associated with superbugs like *E. coli,* and the possible therapeutic approaches against MDR pathogens.

Key words: antibiotic resistance; extended spectrum β-lactamase; horizontal transfer; therapeutic approaches; nanoparticles; bacteriophage; antimicrobial peptides; gene therapy

Introduction

Antibiotic resistance is considered one of the life-threatening hazards to human health in the 21st century, resulting from increasing international movement, greater use of antibiotics in animal production, deprived sanitation, and inefficient sewage and waste removal systems (1). Antimicrobial resistance (AMR) is emerging at a faster rate than available control methods due to the increasing use of antibiotics in human and animal medicine. It is estimated that it will cause 700,000 deaths every year. In addition, by 2050, the number of deaths due to AMR bacterial infections will reach 10 million each year (2). WHO recently announced the global impact of MDR on global health (3).

The development of antibiotics appears to be one of the most important medical revolutions in the history of modern medicine. Its application significantly reduced the morbidity and mortality accompanying bacterial infections (4). The earliest antibacterial drugs developed, including peni-

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cillin and tetracycline, were based on natural compounds formed by environmental microorganisms (5). Later, various synthetic and semi-synthetic antibacterial agents were developed, including macrolides, cephalosporins, quinolones, and aminoglycosides (6). Despite the fact that AMR is a natural defence of bacteria which has occurred for many thousands of years, the extensive use of antibacterial drugs in human and animal medicine has led to faster development (7).

The upgrowing resistance to various classes of antimicrobials is contributed to by bacterial acquisition of resistance and virulence genes. Around 20,000 potential resistance genes are revealed by recent bacterial genome analysis (8). Many pathogenic strains have developed resistance to antibiotics, and some have developed resistance to many currently available antibiotics and chemotherapeutics. Extended spectrum β-lactamase (ESBL) and carbapenemase - producing Gram negative bacteria as carbapenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, ESBL or carbapenem resistant *Klebsiella pneumoniae*, in addition to methicillin and vancomycin resistant *Staphylococcus aureus* (MRSA and VRSA) and vancomycin resistant *Enterococcus faecium* (VRE) are the most encountered emerging pathogens (9).

The emergence of the AMR gene makes some infections completely resistant to all currently available antibacterial drugs. The latest example of a hazard resistance gene is the New Delhi metalo-β-lactamase 1 (NDM1), which encodes an enzyme that confers various antimicrobial resistance in *E. coli*, which was discovered in India and spread due to migration to the UK. (10). Another example of an emerging gene is the gene carried by the plasmid, which confers resistance to colistin and seems to be the last antimicrobial drug, called mobilized colistin resistance gene 1 (*MCR1*), which was originally found in Chinese pigs (11). The presence of the plasmid encoding fimbriae (*pefA*) enhances the ability of *Salmonella* to adhere to epithelial cells and the invasiveness of *Salmonella* isolates. In addition, many important virulence genes, such as *invA*, *stn*, *sopE1*, *pefA*, and *fimH*, along with the pathogenic *Salmonella* serotype, have been identified by PCR (12).

Methicillin-resistant *S. aureus* (MRSA) produces penicillin binding protein (PBP), which can reduce the activity of β-lactam antibiotics. The low binding affinity of PBP2a encoded by the *mecA* gene to the β-lactam antibiotic allows the continuous synthesis of peptidoglycan cell wall in the presence or absence of a lethal concentration of methicillin (13). In this review, we will discuss the emerging patterns of AMR, its mechanism of action, and therapeutic approaches to fight this life-threating issue.

Mechanism of antimicrobial resistance

The antimicrobial action is either bacteriostatic, which renders bacterial propagation impossible, or bactericidal, which causes bacterial lysis or death. They specifically disrupt the metabolism of bacterial cells to prevent their growth and kill them by inhibiting the synthesis of bacterial cell walls as β-lactams and bacitracin, cell membranes as polymyxins, proteins as chloramphenicol, macrolides, aminoglycosides and tetracyclines, nucleic acid as quinolones and rifampicin or inhibiting folic acid metabolism as sulfonamides and trimethoprim (14).

The development of resistance genes is associated with cytotoxic antimicrobial agents inducing certain biochemical activities that prompted the production of a precursor protein as a powerful selection pressure which is responsible for the evolution of the main microbial resistance genes acting with other resistance cryptic genes in the resistome (15).

Various genetic strategies such as chromosomal changes, genetic material exchange through plasmids and transposons are recorded as occurring in *Streptococcus pneumoniae*, *Strept. pyogenes*, as well as in *Staphylococci*, *Enterobacteriaceae*, and *Pseudomonas* families which were found to cause resistance among them (16).

Other biochemical and physiological strategies were recognized as methylation of 23S rRNA, lactone ring hydrolysis, removal of efflux pump, point mutation for inducing resistance (17).

Bacterial AMR can be either of intrinsic or acquired origin. Intrinsic resistance involves the innate ability and inherent properties of all organisms of a certain species to overcome and tolerate either an antimicrobial or a whole antimicrobial class (18). Acquired resistance refers to bacteria that develop resistance to a previously sensitive antibiotic. This may be due to the development of specific selective pressures over time, forcing microorganisms to develop defence mechanisms

(19). Acquired resistance arises from vertical evolution, which includes mutations in regulatory or structural genes within the bacterial genome that are transmitted from parent to daughter cells, such as resistance to fluoroquinolones and oxazolidinones (18, 20).

Horizontal transfer is a means for some bacteria to acquire resistance by exchanging mobile genetic elements such as plasmids, transposons, and integrons, leading to the faster spread of antimicrobial resistance between diverse bacterial species (21).

Spread of resistance between ecosystems

Resistance can spread between ecosystems in two disparate ways: direct way in which resistant bacteria themselves are recognized in another environment (22). Indirect transmission of resistance genes helps to produce new genetic material, which can be produced by transformation, conjugation, or transduction (23).

One of the most common ways to share genes is through Horizontal Gene Transfer (HGT) which includes three main ways of genetic recombination: transformation, transduction, and conjugation. Transformation, where the naked DNA is taken up by the recipient bacteria because they can take DNA from the environment and incorporate it into the host genome through homologous inversion or recombination in both Gram-positive and Gram-negative bacteria.

However, the most common way of exchanging resistance genes between bacteria is conjugation, where the elements of conjugation or mobilization consist of plasmids, transposons or genomic islands.

Bacteriophages also play a part in spreading DNA between bacteria through a process called transduction, in which bacterial DNA is packaged on the head of the phage and injected into the recipient bacteria. A gene cassette is a mobile genetic unit that can exist freely in the form of circular DNA, or can be incorporated into more complex genetic elements called integrons (23). Horizontal integron transfer is facilitated by plasmids or transposons.

Plasmids are linear or circular extrachromosomal mobile genetic elements (MGE) that have their own genes necessary for replication and contain genes for the origin of transfer and coding of functions, allowing them to be transferred to a new host by conjugation. In addition, plasmids often encode antimicrobial resistance genes integrated or not integrated into transposon or integron elements. If a plasmid contains an antimicrobial resistance gene on the transposon, it can be transferred to the bacterial chromosome and maintained without the plasmid, thereby promoting the spread of resistance (23).

Super-resistance associated with superbugs

Due to mutational resistance to various antibiotics, super bacteria cause microorganisms to exhibit high morbidity and mortality. The emergence of MDR bacteria recently represents a serious global public health problem (24).

Treatment measures are insufficient to combat these drug-resistant pathogens and are accompanied by higher financial costs and longer hospital stays. They showed increased virulence, resistance and higher spread rates (8). Some important MDR pathogens include *E. coli, S. aureus, S. epidermidis*, *Mycobacterium*, *Enterococcus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *S. pneumoniae*, *Proteus*, *Serratia*, *Heamophilus*, *Salmonella*, *Burkholderia*, *Clostridium*, *Campylobacter*, and *Salmonella* species (8, 25).

E. coli is a vigorous opportunistic pathogen resulting in various extra-intestinal infections including pneumonia, neonatal meningitis, diarrhea, and urinary tract infection (26). Major resistance genes within *E. coli* were identified like β-lactam resistance genes. β-lactam antibiotics include penicillin derivatives, cephalosporins, monobactams, and carbapenems. Their lethal targets are the transpeptidase domains of penicillin binding proteins (PBPs), that catalyze the crosslinking of bacterial peptidoglycan (PG) during cell wall synthesis. In the presence of β-lactam, peptidoglycan cross-links cannot be formed, making the cell wall unable to withstand changes in osmotic pressure, resulting in cell lysis. β-lactamase shows resistance to β-lactam by hydrolyzing and inactivating the central ring of β-lactam. β-lactamase is divided into A, B, C and D according to the amino acid sequence (27). Class B β-lactamases are metalloenzymes e.g. New Delhi metallo-β-lactamase 1 (NDM-1), class C (cephalosporinases); classes A and D (serine carbapenemases and extended-spectrum β-lactamases; ESBL) that shows resistance to all aminopenicillins, the difference is to third generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and monolactams, aztreonam,

but to cephalosporins or carbapenems not resistant. ESBL have been classified into three major subtypes: TEM, SHV and CTX-M -lactamases, and group 3 metallo-β-lactamases (28). The most common type of plasmid-mediated β-lactamase is TEM, which encodes resistance to ampicillin (29), SHV can also be found on the chromosome and confers broad-spectrum penicillin resistance (30), OXA which nearly always plasmid-mediated and responsible for resistance to penicillins, cephalosporins and carbapenems (31), and CTX-M, which specially hydrolyses cefotaxime (32). These β-lactamases are encoded by the bla gene, which can be found in plasmid and chromosome cassettes (33). In addition to blaTEM and blaSHV, *E. coli* also has the AmpC gene encoding AmpC β-lactamase. blaTEM and blaSHV belong to subclass 1 and are cephalosporinase (34). The AmpC gene is mediated by the plasmid or chromosome; due to the promoter in the mobile element, it is expressed at a higher level in the plasmid (35).

TEM and SHV types were for a long time the dominant ESBL enzymes worldwide, but nowadays, CTX-M enzymes have become the most widespread type of ESBLs (36).

Possible therapeutic approaches to multidrug resistant bacteria

Therapy with nanoparticles

Nanotechnology is a vital approach that includes applying nano-size particles of about 1 to 100 nm. Recently, they are used in different fields such as molecular biology, organic and inorganic chemistry, physics and medicine (37). Nanotechnology has recently been used as a tool to explore potential avenues for therapeutic applications in a variety of ways, including imaging, sensing, targeted drug delivery, gene delivery systems, and artificial implants (38).

Nanoparticles (NPs) are usually between 0.2 and 100 nm, which allows interaction with microorganisms. Identification of antimicrobial properties of NPs, especially metallic NPs, leads to the evolution of several nanostructures (39). Therefore, nanoparticles of metal and metal oxides such as silver, gold, silver oxide, zinc oxide, titanium dioxide, calcium oxide, copper oxide, magnesium oxide, and silicon dioxide are considered antibacterial agents (40). Metal nanoparticles have a wide range of biomedical applications as antibacterial agents due to their unique physical and chemical properties (39).

Mechanism of action of nanoparticles

Nanoparticles are well known to cause extensive gene mutation within the same bacterial host, thus rendering the emergence of microbial resistance later (41). Nanoparticles may act by producing reactive oxygen species (ROS), bacterial membrane lysis by release of metal ions, altering membrane permeability, prevent the biofilm formation as they act as intercalating agents, so they disturb the ribosome destabilization (42). Like peptides, NPS may represent an electrostatic interaction that enables them to bind to the cell membrane, leading to loss of its integrity. All types of NPs generally have the ability to block cell membranes and cause cell death. The nanotoxicity produced by the combination of NP and the surface of the bacteria produces oxidative stress as a redox reaction. It usually accumulates free radicals such as hydrogen peroxide, superoxide anions, and hydroxyl radicals, which lead to cell degradation and inhibition of DNA replication (40). Current metal oxide is used as an alternative to third-generation antibiotics. Recurrent use of metallic NPs may influence their safety because frequent exposure to the same type at sub inhibitory levels may lead to resistance in bacterial pathogens (43). The cytotoxicity of NPs can be determined by physical and chemical characteristics, such as size, configuration, structure, and surface charge (38). The higher concentration of NPs is not recommended in microbial treatment because it may influence eukaryotic cells. On the other hand, NP treatment can cause potential cytoto-xicity, genotoxicity and carcinogenicity, induce apoptosis, and inhibit cell proliferation (44).

Silver nanoparticles in therapy

Silver nanoparticles (AgNP) show great antibacterial activity due to its multi-directional approach that can reduce the development of drug resistance (45). The role of AgNPs is to release Ag + ions, leading to the interruption of the electron transport pathway or the production of ROS, and ultimately destroying important biomolecules, such as cell walls, cell membranes, cell DNA and/or proteins (46). The repeated exposure to AgNP sub-inhibitory levels can cause bacterial pathogens to develop resistance. It has been suggested to use

AgNP in combination with antibiotics to overcome this limitation and increase the therapeutic efficacy. AgNPs have been recognized to show synergistic antibacterial activities against MDR pathogens when applied with different antibiotics as polymyxin B in the treatment of a mouse model infected with *A. baumannii* showed a survival rate of approximately 60% compared to controls treated with antibiotics or AgNP alone (47).

Bacteriophage Therapy

Bacteriophages have been extensively described as biocontrol agents in the food industry as a means of controlling pathogenic bacteria (48). Some phage formulations such as EcoShield™, ListShield™, and Listex P100™ have been recognized for this target and have been effectively used in meat, marine, farm and dairy products (49). Lytic phages, against superbugs, have been isolated from hospital wastewater so that they are considered easily available therapeutic agents (50). Bacteriophages which are used for therapy have many advantageous characteristics such as high host specificity, avoid destroying normal flora, do not infect eukaryotic cells, proliferate rapidly inside the host bacteria, and only require low dosages for treatment. Therefore, they are considered to be ideal therapeutic approaches to cure bacterial infections (51). Phages are recommended over antibiotics as they create new infectivity and destroy bacteria as they mutate along with their host (52).

Recently, phage mixtures composed of phage combinations have been applied to various bacterial species or strains (53). An ideal phage cocktail is revealed to be prepared using different phages of diverse families or groups as a wide range of hosts for the preparation, with a high empathy for all cell wall components in bacteria. The application of such phage cocktails could diminish the development of phage resistant bacterial species or strains (54).

It is a vital concern to recognize the genomic characterization of phages to ensure their safety in therapeutic approaches. Phages may act as vectors for horizontal gene transfer in bacteria. Occasionally they are also involved in transferring virulence or antibiotic resistance genes to develop extra pathogenic or antibiotic-resistant microbes (55). Unfortunately, bacteriophages used for therapeutic applications may contain genes for virulence or antibiotic resistance, as well as integrases and inhibitors of their lytic cycle, which contribute to the appearance and integration of these genes in the host bacterial genome (56). The efficacy of phage therapy in treating patients with eye infections is reported (57), as well as diabetic foot ulcer (58), and urinary tract infections (59). It was reported in a case study of a secondary eye infection caused by VRSA, which was well cleared against MRSA by using the commercially available lytic bacteriophage SATA8505 (ATCC PTA9476) (57).

A strategy has recently been developed to overcome restrictions of phage therapy via phage combination with antibiotics that may show effective synergistic action in reduction of bacterial biofilm formation (60). Furthermore, endolysin produced by bacteriophages has been found to be more valuable than lysing the cell wall and allowing antibiotics to enter the interior of bacteria (61). The combination of bacteriophage PEV20 and ciprofloxacin has a synergistic effect on *Pseudomonas aeruginosa in vitro* (62).

Antimicrobial peptides in therapy

Antimicrobial peptides (AMP) are broad, short, positively charged host defence oligopeptides, produced by all living forms, including protozoa, bacteria, archaea, fungi, plants, and animals (63). They show a diverse means of activity against a wide range of pathogens. Antimicrobial peptides have no effect on eukaryotic cells because the cell membrane is made of phosphatidylcholine and has a neutral charge (64). AMPs interact with bacterial cell membrane, causing lysis of cells, so it is declared to be a potential substitute to fight MDR pathogens (65). The role of AMP is to promote cell lysis through pore formation. First, the electrostatic interaction between the positively charged peptide and the negatively charged bacterial cell membrane further inserts the peptide into the membrane, where it initiates the formation of peptide aggregates, allowing pore formation and subsequent cell lysis (66). Unlike commercial antibiotics, AMPs cause physical damage to bacterial cells through electrostatic interactions, leading to bacterial resistance to AMPs (67). Several natural and bioengineered AMPs have been used *in vitro* and *in vivo* (68). They have antibacterial, anti-inflammatory, anti-

biofilm and wound healing capabilities, and have extremely low cytotoxicity. For example, Histatin 5 is a cationic human salivary peptide that is naturally rich in histidine. The peptide showed strong anti-biofilm and effective bactericidal activity against MDR pathogens (≥ 70%) *in vitro* (69). Compared with antibiotics such as tobramycin, ciprofloxacin, ceftazidime and vancomycin under 1X MIC, the newly designed cationic peptide WLBU2 and natural AMP LL37 inhibit biofilm under 1/3X MIC The rate is 90% (70). Recombinant peptides derived from human ApoB were examined, especially r (P) ApoBL and r (P) ApoBS. It exhibits anti-inflammatory, anti-bacterial and anti-biofilm wound healing properties in vitro and can resist MDR strains of S. aureus and *P. aeruginosa* (71). Human lactoferrin peptide HLR1r in very low concentrations was found to show anti-infective, anti-inflammatory and non-cytotoxic effects in wound excision models in rats infected with MRSA, indicating that HLR1r is used in topical preparations for the treatment of skin infection (72). Peptide T13, is a peptide derived from the seeds and leaves of the crude extract of Populus tomentosa. It has also shown effective in vivo antibacterial activity in the wax moth model of *S. aureus* infection (73). Synthetic FeleucinK3 analogs have been shown to eliminate Pseudomonas aeruginosa-induced bacteremia in a mouse model, with good stability and extremely low cytotoxicity (74). In addition, hydrogel formulations containing K11 (a hybrid peptide of melittin, cecropin A1, and magainin2) have shown wound healing ability in mouse excision models of *A. baumannii* infection, indicating that it can be used as a topical therapy (75). The few challenges that hinder the *in vivo* efficacy of AMPs include their cytotoxicity to mammalian cells, the possibility of cathepsin degradation, loss of activity in the presence of low salt concentrations or plasma proteins, and increased production costs (76). This can be avoided by modifying the AMP, such as adding unnatural amino acids or their isomers, peptide cyclization, acetylation, and N-terminal amidation. The use of suitable delivery systems such as liposome encapsulation can improve their stability and reduce toxicity (77). The combination of AMP with antibiotics (71, 78) or nanoparticles can increase the efficiency of AMP (79). The synergistic effects of A3APO, a proline-rich AMP, and colistin in a mouse model of bacteremia infected by *K. pneumoniae* were also reported (80). When LL37 (a human cathelicidin peptide) is used in combination with gold nanoparticles, the *in vitro* bactericidal activity against *S. aureus* is higher than when vancomycin is used alone (81).

Photodynamic light therapy

Antimicrobial phototherapy alone or in combination with photosensitizers (PS) can respond to photooxidative stress that leads to the death of microorganisms. Excitation of PS with an appropriate wavelength of light produces an excited triplet state, which can transfer electrons or energy to biomolecules or molecular oxygen, resulting in the formation of ROS or singlet oxygen free radicals, which are toxic to cellular targets such as nucleic acids, proteins, and lipids (82). The most widely used PS are phenothiazine derivatives (methylene blue, toluidine blue), xanthine derivatives (rose Bengal), porphyrin, chlorin or fullerene derivatives, etc. Antimicrobial photodynamic therapy is widely used to treat tooth, skin, and soft tissue infections (83). The highest PS used for antibacterial therapy is characterized by increased permeability to microbial cell walls or membranes, selective toxicity to microbial cells, no effect on host tissues, and adequate absorption factors for effective penetration at the site of action. The selected PS should not have a long half-life, which will lead to long-term photosensitization in the host cell even after the infection has been cured. Furthermore, it should not be excreted by the microbial efflux system (83). The effectiveness of Photo Dynamic Therapy (PDT) also depends on luminous flux, PS concentration, and processing time (84). *In vitro* studies have shown that blue light (aBL) has broad-spectrum antibacterial and antibiofilm activity against all six members of *Enterococcus faecium*, *S. aureus*, *K. pneumoniae, A. baumannii*, *P. aeruginosa*, and *Enterobacter* species (ESKAPE) are the principal cause of nosocomial infections throughout the world (85). Combination with nanoparticles is used to allow PS to enter through the membrane or to cause a coordinated ROS reaction leading to bacterial cell death (86). Silver NPs were also used combined with blue light

showing synergistic antimicrobial and antibiofilm activities against *P. aeruginosa* infection (87).

Recently, the synergy of phototherapy with antimicrobial peptides, antibiotics, nanoparticles or efflux pumps has also been recognized (88).

Vaccines

Several methods, especially genetic engineering and bioinformatics tools, have been established to identify antigens for preparing vaccines that enable the recognition and production of epitopes that in turn activate the immune response. These vaccines consist of at least one adaptation antigen, which when administered with an adjuvant, induces an immune response against the pathogen. In addition, live modified microorganisms such as *Listeria monocytogenes, Mycobacterium bovis, Salmonellae* spp,. and*, Shigellae* spp. They are used in certain vaccines. In addition, the application of genes encoding antigens together with vectors can induce the synthesis of specific antibodies against pathogens (89, 51). Vaccines used for the inhibition of typhoid fever include, for example, Ty21a which is a live attenuated oral vaccine present as enteric capsules and parenteral Vi vaccine (90). There are many approaches to developing upgraded live, attenuated, and oral vaccines including safety, efficiency, and reducing the number of doses required (91). The efficiency as well as preparation of vaccines has decreased with the evolution of MDR pathogens as they continue to change their antigenic nature (92).

Applying gene therapy

New gene therapy may be the alternative method of choice to compete with the MDR strains as it may induce genetic modification and inhibit virulence. In general, all bacteria respond to stressful conditions by changing their gene expression pathways (93). In order to change gene expression, a sensor that can detect precise chemical or physical signals and change the level of related regulatory proteins is needed. In addition, it causes changes in cell composition, which helps bacteria control stress conditions. PhoP-PhoQ, a better-characterized two-component regulation system, can manage a variety of physiological functions, mainly virulence and mediate adaptation to Mg2 + restricted environments. PhoQ is an inner membrane protein that can phosphorylate and dephosphorylate the DNA binding protein PhoP in response to environmental fluctuations. The PhoP-PhoQ system responds to the intensity of Mg2+ and Ca2+ concentrations (94). The decrease of Mg2+ concentration improves the phosphorylation of PhoP protein and the transcription of PhoP-activated genes (95), while high Mg2+ suggests dephosphorylation and prevents PhoP-activated genes expression (96). The phosphorylation of PhoP protein supports the structural change of its C-terminal DNA binding domain, thereby enhancing the attraction of PhoP protein to its promoter. The regulatory components of PhoP-PhoQ are thought to control the virulence of *Salmonella*, which in the late 1980s triggered new attention to the two-component regulatory system (97). The incidence of the PhoP/Q system in various bacterial groups indicates that PhoP can control the expression of genes in different species (98).

Conclusion

Antimicrobial resistance is a growing threat to human and animal health. It reduces the ability to treat bacterial infections, thus reducing the risk of morbidity and mortality caused by resistant bacteria. There should be a global prohibition on antimicrobial use in agriculture to conserve their effectiveness in treating human diseases. Ensuring the efficacy of antimicrobials to treat bacterial infections remains a persistent concern either for veterinary or human medicine. The search for new antimicrobial drugs to combat infections caused by drug-resistant pathogens is currently growing. Research groups around the world are suggesting alternative solutions for the treatment of drug-resistant organisms, and it is easy to speculate on the post-antibiotic golden age characterized by the use of biological, biological or bio-based products and therapies. Current advances in biotechnology, genetic modification, molecular biology, and advanced chemistry have led to the discovery of various antibiotics from the environment, insects and other animals, fungi, and plants. Several therapeutic approaches like vaccines, antimicrobial peptides, bacteriophages and nanotechnology, along with phytochemicals and herbal medicines, are applied *in vitro.* Hence, *in vivo* experiments are supposed to be suitable candidate for antimicrobials to combat the rapidly evolving AMR.

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