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CORRESPONDENCE ARTICLES

Colistin Resistance Gram-Negative Bacteria as an Emerging Public Health Threat

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ABSTRACT

The increasing resistance of multidrug-resistant (MDR) bacteria has become a major threat to public health and has led to the use of polymyxins, with their toxicity, as a last resort in the treatment of infections caused by multidrug-resistant bacterial strains. Currently, two polymyxins are available, polymyxin E (colistin) and polymyxin B. In the mid-1990s, Colistin was introduced as a treatment for infections caused by MDR Gram-negative bacteria (GNB). Unfortunately, resistance to colistin has rapidly emerged after its introduction into treatment protocols against these resistant strains. Considering this, our study focused on the recently discovered mechanisms of colistin resistance, starting from China and then other countries around the world to elucidate the mechanisms of colistin resistance and track the spread of colistin-resistant bacterial strains, which would provide essential information on various aspects of the increasing prevalence of colistin-resistant bacteria. In this context, this review highlights the progress made over the past two decades in understanding the mechanisms of action of colistin and the different strategies used by bacteria to develop colistin resistance, as well as provides an update on what was previously known and what is new, to evaluate the monitoring colistin resistance and identifying resistance trends which deserves further study and development of future strategies to control the spread of multidrug-resistant pathogens.

INTRODUCTION

The escalating increase in resistance of antibiotics over the past 50 years has led to a global crisis in the treatment of pathogens, especially Gram-negative bacteria[1]. The uncontrolled clinical use of antibiotics has also led to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and completely drug-resistant organisms (PDRO) such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumoniae*, causing therapeutic challenges led to high morbidity and mortality. Consequently, therapeutic challenges have prompted clinicians to reuse colistin (polymyxin E), an older antibiotic as a last resort drug for treatment of those infected patients with bacterial infections caused by resistant bacterial strains[2].Two polymyxins are available, polymyxin E and polymyxin B, are polypeptide antibiotics isolated from Bacillus polymyxa, discovered by Koyama in Japan in 1947[3]. Polymyxins includes five chemical compounds (polymyxins A, B, C, D, and E)[4]. These chemical compounds, mostly act by disrupting the bacterial cell membrane[5]. The chemical structure of colistin is a cyclic heptapeptide with a tripeptide side chain acylated at the amino terminus by a fatty acid tail, this hydrophobic tail is responsible for the antimicrobial property in colistin, as well as its toxicity[6]. Polymyxins B and E are used clinically in two forms: intravenously or intramuscularly [7]. The first use of colistin in human medicine was in the 1950s as an intravenous formulation[8], however, colistin has been used recently been revived in humans as a last resort against infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB). Colistin was approved by the United States (US) Food and Drug Administration (FDA) as an antibiotic in 1959 to combat infections that caused by GNB. Therefore, it was used to treat urinary tract infections (UTIs) and infectious diarrhea. Polymyxins have also been used for years to combat infections caused by Gram-negative bacteria, and for decades in topical formulations for selective bowel cleansing and the treatment of ear and eye infections[9]. In 2012, the World Health Organization (WHO) declared colistin an essential antibiotic for human medicine[10]. The European Medicines Agency (EMA) also considered colistin is a class B antibiotic use in human therapy[11]. The clinical misuse and overuse of colistin widespread, led to emergence of colistin resistance. Unfortunately, many Gram-negative bacteria have genes resistant to colistin and that help the bacteria to become resistant to the colistin [12]. World Health Organization (WHO) in 2017, issued a list of antibiotic-resistant pathogens, that was categorized as critical, high, priority pathogens[13]. It was believed that chromosomal mutations in the genes encoding the

regulate negative regulatory systems MgrB or the PmrA/PmrB and PhoP/PhoQ two-component systems (TCS) are the cause of colistin resistance[14]. However, plasmid-mediated mobile colistin resistance gene (mcr-1) discovered in China in 2015 was identified in *Escherichia coli* isolates and has been shown to confer colistin resistance in some strains of Gram-negative bacteria [15]. Subsequent studies have reported the mcr-1 gene on a global scale in *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*[16]. A study conducted during the years 2016 to 2021 reported that *E. coli* is the predominant species that contain plasmid-dependent mobile colistin resistance genes (mcr) and its horizontal transferability[17]. To date, more than 25 different variants of the mcr-1 gene have been determined[18]. Therefore, Gram-negative bacteria use a wide range of mechanisms to develop resistance to last-resort drugs, which is a major cause for concern and complicates treatment options for these bacterial infections[19]. The review aims to highlight the resistance of Gram-negative pathogens to colistin and discuss the progress made in understanding the mechanisms of this emerging resistance and future prospects for its reduction.

MECHANISM OF COLISTIN ACTIVITY

The direct target of action of colistin against GNB is the outer cell membrane. GNB are characterized by the presence of lipopolysaccharide (LPS) in the outer membrane (OM), which contributes to the integrity and stability of the bacterial membrane and prevents the entry of antibiotics and hydrophobic ingredients[20]. The lipopolysaccharide structure consists of three domains: a core polysaccharide, a conserved lipid A that acts as a hydrophobic anchor to the OM, and an O antigen chain[21]. When anionic lipid A binds to cationic colistin a major component of LPS occurs for the GNB electrostatic interaction, causing the displacement of magnesium (Mg2+) and calcium (Ca2+) ions from LPS. This resulted in the inactivation of outer LPS and subsequent loss of cellular contents, thus killing the bacteria, a bactericidal effect [22]. Indeed, LPS is the primary target for killing bacterial cells, but the exact mode of action of colistin is still uncertain. Several studies have suggested that disruption of the outer and inner GNB membranes is the most important mechanism of action of colistin. There are also other mechanisms of action of colistin against Gram-negative bacteria, including inhibition of respiratory enzymes, which is a secondary mechanism of colistin activity. The bacterial respiratory chain consists of three complexes containing adenine reductase, nucleotides, and quinones, which primarily transfer protons and electrons between protein complexes[23]. In addition, colistin inhibits the activity of the endotoxin lipid A fraction of GNB by binding to and neutralizing lipopolysaccharide molecules. Although polymyxins show less activity against Gram-positive bacteria due to their lack of binding to lipoteichoic acid in the cytoplasmic membrane, polymyxin B has been found to be effective against Gram-positive bacteria .The inclusion of hydrophobic functional groups in the structure of polymyxin B through fatty acids may increase its membrane-penetrating ability and lead to interactions with lipoteichoic acid in Gram-positive bacterial membranes, which in turn increases its antibacterial activity[24].

COLISTIN HUMAN USE

Emergence of antibiotic-resistant pathogens had led to a scarcity of treatment options for patients, which has led to the introduction of colistin into treatment protocols for patients as a last resort for the treatment of bacterial infections caused by multidrug-resistant Gram-negative bacteria. In 1950, colistin was first used as an intravenous injection, and the US Food and Drug Administration approved it in 1959 for use as an antibiotic, due to its effective effect in eliminating gram-negative bacteria[25]. Colistin has been used to treat chronic lung infections caused by *Pseudomonas aeruginosa* in cystic fibrosis patients aged six years and older, in addition to treat bacterial infections caused by Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, Enterobacter species, and Acinetobacter species. Colistin is available as an intramuscular and intravenous injection, where the powder is mixed with water at a concentration of 150 mg. It is also used as a spray for inhalation through a nebulizer[26]. Colistin is a positively charged cation polypeptide that binds to phospholipids on the cell membrane of Gram-negative bacterial cells, which changes the osmotic barrier of the membrane, resulting in the leakage of the bacterial cell's contents of metabolites and nucleosides, causing the death of the bacterial cell[25]. When using colistin with some medications such as, Vancomycin, aminoglycosides, Amphotericin B, and Capreomycin, cause kidney or neurotoxicity. Unfortunately, due to the high rates of renal and neurotoxicity caused by colistin, it has been discontinued over the past years. Recently, due to, increasing rates of bacterial infections caused by multidrug-resistant Gram-negative bacteria worldwide, the clinical use of colistin has recently returned as a last resort treatment option for emerging serious bacterial infections[26]. Indeed, the use of intravenous colistin has gone through three stages. The first stage: from the fifties to the seventies, where it was used as a treatment for infections caused by Gram-negative bacteria in general. Second stage: last ten years of the last century, it was used as a treatment for bladder infections resulting from infection with Gram-negative bacteria that are multidrug-resistant. Third stage: from 2000 until

now, it has been used against infections resulting from multidrug-resistant-Gram-negative pathogens[27]. Of note, several international consensus recommendations have also directed the controlled and appropriate clinical use of colistin, which represents the last line of defense against multidrug-resistant carbapenemase-producing Gram-negative bacteria[28].Moreover, some Gram-negative pathogens are not sensitive to colistin, such as Neisseria, *Moraxella catarrhalis*, Serratia, *Helicobacter pylori*, Brucella, and Proteus. However, colistin can be used in critical clinical conditions such as pneumonia in the intensive care unit. Furthermore, colistin is considered an alternative treatment for many clinical conditions, such as osteomyelitis, arthritis, urinary tract infections, gastrointestinal infections, abscesses, and eye and ear infections. However, conditions in which colistin is contraindicated are myasthenia gravis and polymyxin hypersensitivity, but there is no scientific data to support this. Thus, colistin should be given to patients with impaired renal function under close monitoring with dose adjustment due to its nephrotoxicity[29]. Recently, studies have shown the use of colistin in the treatment of meningitis due to its permeability through the blood-brain barrier and its use in the treatment of bladder and urinary tract infections such as *Acinetobacter baumannii*. Moreover, in intensive care units, colistin is used orally to decontaminate the gastrointestinal tract and reduce bloodstream infections and mortality, but over time, an increase in mortality due to colistin resistance has been reported among *Klebsiella pneumoniae* isolates [30].Unfortunately, the extensive use of colistin in treatment protocols has led to the emergence and spread of high colistin resistance among multidrug-resistant Gram-negative bacteria, a critical health issue that is currently of widespread concern worldwide.

COLISTIN RESISTANCE MECHANISM

Colistin resistance can occur through several diverse mechanisms, such as a chromosomal mutation due to the modification of lipid A moiety of LPS, as observed in bacteria with resistance to polymyxins. This is because any change in LPS can change the behavior of colistin. These mutations decrease the negative charges of the LPS membrane by shielding carboxyl groups and phosphate to prevent colistin action and binding[31]. Bacteria have developed resistance mechanisms to colistin by reducing its negative charge of the LPS. This can be achieved by the incorporation of 4-amino-4-deoxy-l-arabinose (l-Ara4N) and phosphor ethanol amine (PEtN) and 4-amino-4-deoxy-l-arabinose (l-Ara4N) to the phosphate groups of lipid A. As a result, the positive charge of LPS increases, reducing its affinity for colistin[32].Bacterial resistance to colistin is resistance to other types of antibiotics, such as β-lactams, quinolones, aminoglycosides, sulfonamide, third-generation cephalosporins, tetracycline, and lincosamides which involve different mechanisms of resistance such as efflux, enzymatic, impermeability, or point mutations. Each genus in the family Enterobacteriaceae has its own different mutation type and characteristic genes associated with colistin resistance. Such as in the genus *E. coli*, mutations happen in the sensor kinases more frequently than in TCS response regulators [33]. *E. coli* and *Salmonella spp*. are able to change lipid A by altering LPS through phosphor ethanol amine (PEtn) and the biosynthesis of 4-amino-4-deoxy-L-arabinose (L-Ara4N). Other mechanisms like the loss of lipid A, OM remodeling events, capsule formation, and efflux pumps have been suggested in Enterobacteriaceae. Its biosynthesis is associated with chromosomal-mediated resistance and is dependent on sensor kinase systems: PmrA/PmrB and PhoP/PhoQ and two-regulators of component response[34]. Two-component systems (TCS), PmrAB, and PhoPQ and their regulatory gene in Enterobacteriaceae are responsible for resistance. A system PmrA/PmrB controls the pmr HIJKL Moperon, which neutralizes the phospholipids negative charge of the cell membrane as the resistance mechanism by *Pseudomonas aeruginosa*[35]. *Acinetobacter baumannii* resistance to colistin is related to the absence of LPS production; this suspension of LPS production may be related to the inactivation of a lipid A biosynthesis gene due to the absence of lipid A. Regarding the resistance expressed by *Klebsiella pneumoniae* to colistin, its resistance mechanism is based on the inactivation of the mgrB gene due to deregulation, regulatory systems PmrAB and PhoPQ 36. It was believed for a period of time that resistance to colistin was due to chromosomal genes (pmrAB, phoPQ, and mgrB) until the plasmid-mediated mcr-1 gene was identified in *Escherichia coli* in China in 2015[37]. Later, other discoveries of this gene were announced in Europe, Asia, America, and Africa[38]. Then followed that, discovered a new colistin resistance gene, mcr-2, in Belgium carried by a plasmid in *E. coli* isolates from biological samples of bovine and porcine, while genus Salmonella carry only the single plasmid mcr-1 gene[39]. Meanwhile, have been identified seven mcr homologues (mcr-3 to mcr-9) in Enterobacter ales [40]. The mcr gene has also been detected in 72 developed countries, and ten mcr genes have been described in Enterobacteriaceae, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella spp*.[41]. This discovery changed the scenario of polymyxin resistance, due to the high rapid spread of this gene and the possibility of horizontal transfer among Gram-negatives, to become a concern limiting the available therapeutic options. Additionally, possible causes of colistin resistance include its long-term use in veterinary medicine and its overuse in human medicine to treat highly resistant bacterial infections[42].

Table 1: Characteristics of colistin resistance mechanisms with most common Gram-negative bacteria

Family	Bacteria	Resistance mechanisms	References No.
Enterobacteriaceae	*E. coli*	Modification of lipid A by activation of the pmrHFIJKLM operon/activation of pmrAB by pmrD	43
		Modification of lipid A by arnBCADTEF operon, pmrC and pmrE genes	44
		Overexpression of phoPQ and activation of pmrHFIJKLM	45
		Efflux pump	46
		Phosphoethanolamine transferase	47
	Citrobacter species	Phosphoethanolamine transferase	48
	Enterobacter Species	Modification of lipid A by activation of the pmrHFIJKLM operon/activation of pmrAB by pmrD	49
		Modification of lipid A by pEtN and l-4AraN	50
		Overexpression of phoPQ and activation of pmrHFIJKLM	51
		Phosphoethanolamine transferase	52
	k. pneumoniae	Modifications of the LPS moiety	53
		Overproduction of capsular polysaccharide	54
		Membrane fluidity/permeability	55
		Efflux pump systems	55
Pseudomonadaceae	*P. aeruginosa*	Modifications of the LPS moiety Loss of LPS	53
		Efflux pump systems Unclear	54
Moraxellaceae	*A. baumannii*	Modifications of the LPS moiety	53
		Loss of LPS	55
		Membrane fluidity/ permeability	55
		Efflux pump systems	55

COLISTIN SIDE EFFECTS

Although colistin has been widely used in the treatment of patients infected with life-threatening pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, the adverse toxic effects resulting from its use in the treatment of these patients have limited its reliance in the treatment of patients. Side effects of colistin use in treatment protocols include hepatotoxicity, nephrotoxicity, and neurotoxicity. Nephrotoxicity causes kidney damage due to increased cell permeability and influx of anions and cations in addition to water molecules, leading to cell lysis. Indeed, the prevalence of nephrotoxicity among adult patients reached 26.7% [56]. Additionally, patient characteristics such as gender, age, and the nature and severity of the disease increase the side effects of colistin, which increases mortality rates. However, colistin toxicity can be reduced by applying a combination therapy strategy that includes colistin with other less toxic antibiotics such as meropenem. Several studies have shown that synergism between colistin and an antibiotic such as meropenem has been shown to be successful in vitro, but this does not necessarily translate into clinical trial results. Another study was conducted on patients with multidrug-resistant *Klebsiella pneumoniae* infection, where colistin was combined with meropenem and given to one group of patients while the other group was given colistin alone. The result was that the mortality rate in the group that underwent the combination therapy was 2.6 times lower compared to the group that was treated with colistin alone, while the rate of renal toxicity did not change significantly in the two groups [57].

FUTURE PROSPECTS FOR COLISTIN RESISTANCE

The resistance to polymyxins has shown different mechanisms of resistance. New mechanisms have also been discovered in resistant bacterial strains. However, there are still unknown mechanisms for some resistant bacterial strains that require further study. In addition, some bacterial species have intrinsic resistance to colistin such as the presence of LPSs modified with LAra4N[58]. Studies show that colistin resistance often occurs after exposure to colistin. However, it has been found that colistin resistance can develop without prior exposure to colistin, which poses a serious threat that hampers the use of colistin as a last resort against

multidrug-resistant GNB. Therefore, studying this phenomenon is of great importance for future protection against the emergence of bacterial strains with new mechanisms of resistance to colistin. The increasing resistance of pathogenic organisms to many antibiotics, especially colistin, has become a major health problem today and must be addressed collectively at the global level. Colistin resistance can be combated by adopting different strategies of drug repurposing, nano-based and CRISPRi-based strategies, colistin combination therapy with other drugs such as colistin and meropenem, photodynamic therapy, and Bacteriophages for combating multidrug resistant Gram-negative bacteria[59-61], therefore, detection of colistin-resistant strains in the laboratory has become crucial to determine its use in treatment protocols. In addition, in vitro colistin susceptibility testing using broth microdilution (BMD) method to determine the minimum inhibitory concentration (MIC) of polymyxins has become fraught with undeniable complications, which are numerous, due to the low diffusion of colistin in agar and its cationic properties. Rapid, easy, standardized and low-cost susceptibility tests have become a requirement and a matter of great importance for their integration into clinical laboratories, especially since several of these methods have been reported as promising for future implementation to obtain reliable results, including the phenotypical methods, such as the Rapid Polymyxin NP Test for screening for laboratories with few technological resources, and modern systems, such molecular tests, and MALDI-TOF, Sensititre ®, to confirm results and understand the colistin resistance and building future prospects for combating colistin resistance [62].

CONCLUSION

Polymyxins have been used for long periods in the past to treat multidrug-resistant Gram-negative bacteria, but their use was discontinued due to their toxicity. After that, colistin was reintroduced into clinical practice which played an important role widely in the treatment of MDR GNB infections. However, colistin resistance patterns have emerged in GNB isolates responsible for this resistance, involving the complete loss of lipopolysaccharide (LPS) due to mutations in the genes. These mutations disrupt lipopolysaccharide biosynthesis, which lead to the bacterium become less susceptible to the antibiotic. Additionally, modification of the bacterial mucosa, Lipid A structure, such as the addition of galactosamine, or phosphoethanolamine (PEtN), decrease colistin's binding affinity, by overexpression of chromosomal pmr CAB operon genes, and acquisition plasmid-encoded mcr genes through horizontal gene transfer. Other resistance mechanisms involve alterations in the outer membrane permeability of a bacterial cell, expulsion of colistin by heteroresistance and efflux pumps. While it is known that the resistance occurs due to bacterial exposure to suboptimal polymyxin doses in treatment protocols, it has been shown that colistin resistance can occur without prior colistin by the acquisition of mcr genes through horizontal gene transfer, this highlights the hypothesis that this resistance may have emerged in non-clinical isolates and was transmitted to clinical isolates, which represents a major threat that may hinder the use of colistin as a last resort against multidrug-resistant GNB if not addressed properly. Therefore, understanding this phenomenon is crucial to protect against the possibility of the development of resistant strains in the future and provide insights into managing antibiotic resistance. Moreover, the challenges of colistin susceptibility tests are undeniable and the development of standardized, rapid and low-cost susceptibility tests in clinical microbiology laboratories in the future of paramount importance, especially phenotypic methods, such as drop Test, and Rapid Polymyxin NP and modern systems, such as molecular tests, and MALDI-TOF, Sensititre® to confirm the results and understand colistin resistance where that colistin still playing important a role in synergistic combination therapies. There are still many unknown resistance mechanisms that need further studies and research to reveal their role in this resistance, which will help us understand how to overcome colistin resistance by developing less toxic and more effective polymyxin derivatives to address emerging and threatening challenges to public health.

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