OPEN ACCESS

Molecular Medicine Communications
DOI: 10.55627/mmc.004.001.0504

Review Article

A Comprehensive Review of Antimicrobial Resistance against Colistin and Countermeasures

Muhammad Usama Shahzad, Laraib Badar, Usman Shareef*

Shifa College of Pharmaceutical Sciences, Shifa Tameer-e-Millat University, Islamabad 44000, Pakistan
***Correspondence**: usman.scps@stmu.edu.pk

Abstract

In times of increasing antimicrobial resistance (AMR), colistin is used as a last resort against numerous gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Unfortunately, recent studies have shown that some species of gram-negative bacteria are becoming resistant to colistin. This has led to an increase in antimicrobial burden and limited treatment options against bacterial infections. This review highlights emerging studies aimed at optimizing the use of colistin. The frequent use of colistin for the treatment of infections has led to colistin resistance. Resistance patterns include intrinsic and acquired resistance mechanisms. The intrinsic resistance mechanism encompasses genetic modification of Lipopolysaccharide (LPS), whereas the acquired resistance mechanism involves modifications of PmrAB and PhoPQ, which alter the LPS membrane. Mutations in the *CrrB, ramA, romA, ramR, and KpnEF genes*, transfer of the *MCR* gene via plasmids, activation of efflux pumps, and capsule formation all accentuate colistin resistance. Various methods have been developed to detect resistance. Different combinatorial therapies and supramolecular traps have also been reported to be efficacious. Moreover, newer strategies and methods are required to prevent this global dilemma. This review comprehensively explains colistin resistance, its mechanisms, detection techniques, and future implications.

Keywords: Antimicrobial resistance (AMR), colistin, gram-negative, lipopolysaccharide, combinatorial therapies.

1. Introduction

Gastric carcinoma Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, fungi, viruses, and parasites acclimatize and flourish in the presence of previously used first-line medications (Morrison and Zembower 2020). It is correlated with an augmented risk of treatment failure and recurrence of disease. It becomes difficult to treat the infection which eventually spreads and causes severe illness and death. AMR is a great threat to our healthcare. Infections with AMR that cannot be treated lead to chronic illness ultimately prolonging hospital stays and escalating healthcare costs (Dadgostar 2019). Targeted therapy and selection of the appropriate antibiotic for the specific infection might slow down the spread of multidrug-resistant organisms (MDR) (Septimus 2018).

Colistin, a bactericidal polymyxin antibiotic is a cationic polypeptide that acts by disrupting the permeability of the cell wall by binding to anionic LPS and displacing divalent cations, i.e. Mg+2 and Ca+2, from the phosphate group of the lipid membrane, eventually causing leakage of cellular contents and subsequent bacterial death (Biswas et al. 2012). Colistin is used for infections caused by multidrug-resistant (MDR) gram-

negative bacteria (GNB) such as *Acinetobacter baumannii(Cai et al. 2012)*, *Pseudomonas aeruginosa (Cortez et al. 2008)*, and *Klebsiella pneumonia (Petrosillo, Taglietti, and Granata 2019).* In the current therapeutic scenario, Colistin is used as the last resort against MDR and pan drug-resistant (PDR) gram-negative infections (A. Z. Bialvaei and Samadi Kafil 2015a). Colistin has been marketed for 50 years as a prodrug colistin methane sulfonate (CMS). Colistin has been used as a salvage therapy and a restricted option for infections caused by MDR gram-negative bacteria. Colistin was used intravenously or through inhalation to treat infections with *P. aeruginosa* in adult and pediatric patients suffering from cystic fibrosis and *MDR A. baumannii* ventilator-associated pneumonia (VAP). To decrease systemic exposure and adverse effects of colistin, it was administered directly to the infected site to achieve selective targeting. The intrathecal administration of CMS to manage CNS infections has also proven to be effective. CMS is a prodrug that upon entering CSF is changed to colistin through hydrolysis. The concentration of colistin in CSF is appropriate through the intrathecal route. Published clinical data, pharmacokinetic profiling, and experimental data have declared colistin to be safe and efficacious in combating CNS infections (Imberti, Iotti, and Regazzi 2014). Intraventricular administration of CMS is efficacious for ventriculitis caused by MDR *A. baumannii*, This treatment proved to be remarkably pertinent with a few reports of chemical ventriculitis, chemical meningitis, and seizures in some patients (Nation and Li 2009).

Currently, different species of certain GNB are developing resistance to colistin. There are different mechanisms by which bacterial species develop resistance against colistin, which include intrinsic, mutations, or adaptation mechanisms apart from acquired resistance via the *MCR-1 gene,* which affects the membrane permeability of the bacteria cell wall (Moffatt et al. 2010) and modification of cell wall components (Snitkin et al. 2013); resistance acquired through heteroresistant bacteria (Jeannot, Bolard, and Plesiat 2017); efflux pumps-mediated Col-R (Cheah et al. 2016); and plasmid-mediated resistance (PMR) to polymyxins (W.G. Lima et al. 2018).

The emergence of Col-R has caused an increase in the burden of antimicrobial infections. It is also becoming difficult to treat the infections caused by those species of bacteria that have developed resistance against colistin, as it is the last resort treatment option. Col-R is increasing the concern of microbiologists for controlling bacterial infections. Until now, *K. pneumoniae (Cannatelli et al. 2014), A. baumannii (Hood et al. 2013), P. aeruginosa (Muller, Plésiat, and Jeannot 2011), S. enterica (T. Lima, Domingues, and Da Silva 2019), V. cholera,* and certain species of *E. coli* have been resistant to colistin. This is becoming a serious issue that needs to be addressed as soon as possible.

In this article, we will discuss the mechanism, susceptibility factors, current situation of Col-R in the world, techniques used to detect resistance in individuals, the ways by which we can slow down further resistance in microbes, and how we can deal with this problem.

2. Colistin - An Effective Molecule Against Resistant Bacteria

The increase in AMR will become a global crisis in the future. There is a need for an antibiotic that can be used against multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) strains of different bacteria that cause hospital-acquired infections in patients. For this purpose, colistin

(polymyxin E), and polymyxin B are used as "last resort" antimicrobials against infections caused by MDR GNB (Watkins, Smith, and Bonomo 2016). Polymyxins are a structurally distinct class of nonribosomal, oligopeptide antibiotics, that are structurally divided into five chemical classes (i.e., polymyxin A, B, C, D, and E) out of which polymyxin B and E are only commercially available (El-Sayed Ahmed et al. 2020).

The LPS constituents in the outer membrane of bacteria provide a protective function, serving as a barrier for large hydrophobic antibiotics (Vidaillac, Benichou, and Duval 2012). LPS is a polyanionic prodrug that serves as the initial target for colistin. Colistin detains a positive charge that alters bacterial membrane integrity and stability. (Abed Zahedi Bialvaei and Samadi Kafil 2015b) Lipid A in LPS plays a pivotal role in membrane permeability and cellular exchange, it also acts as an endotoxin. (Vidaillac, Benichou, and Duval 2012) Colistin through electrostatic interaction binds to the negatively charged phosphate group of Lipid A, a component of anionic LPS. By binding to Lipid A, it exhibits anti-endotoxin activity. These endotoxins are microbial mediators in case of septic shock (Sampson et al. 2012). Colistin microspheres decrease these endotoxins by neutralizing them and by inhibiting cytokines release in endotoxin-induced sepsis. The Cationic detergent activity of colistin is proof of its bactericidal activity, which occurs via a two-step mechanism. It displaces Ca+2 and Mg+2 hence deranging the LPS constituents of the cell membrane. These divalent ions are membrane stabilizers that are competitively displaced by colistin. This results in altered permeability of the cell membrane and osmotic gradient of the cell, thus impairing the three-dimensional LPS framework (Mendes and Burdmann 2009). The second step involves the incorporation of a terminal acyl fat chain constituting a hydrophobic nature, it augments permeation of colistin into the inner membrane. This allows colistin to interact with the inner cytoplasmic membrane (Li et al. 2005). The hydrophilic groups in fatty acid chains cause the phospholipid bilayer of the inner member to lose its integrity. This results in havoc eventually causing cell lysis and discharge of cellular components (Abed Zahedi Bialvaei and Samadi Kafil 2015b). **Figure 1** represents the mechanism of action of colistin. It demonstrates how colistin kills bacteria and shows its antibacterial effects.

3. Epidemiology of Colistin Resistance

Resistance to colistin has been detected in various parts of the world. Certain GNB mentioned in **Table 1** have developed resistance to the drug. According to recent reports, in Pakistan, *A. baumannii 27, K. pneumonia 28,29 & E. coli 30,31* have become resistant to Colistin. Reports also show a high prevalence of resistance against Colistin in tertiary care hospitals (Bashir and Ahmed 2016; W. Liao et al. 2022). There also have been an increasing number of reports of Col-R from different regions of Europe (Y. Wang et al. 2020), Korea (S.-Y. Lee et al. 2020), Lebanon (Moghnieh et al. 2021), USA (Tyson et al. 2020) & Russia (Kuleshov et al. 2021; Azizov et al. 2019). Thailand has the highest prevalence of resistance to Colistin while South Korea reports the least resistance (Uzairue et al. 2022).

4. Mechanistic Insights in Colistin Resistance

The following resistance mechanisms are demonstrated in **Figure 2**. It briefly describes how certain species of bacteria are developing resistance against Colistin.

Table 1 shows the Antimicrobial Spectrum of Colistin & its Resistance Pattern throughout the world.

Name of bacteria	Resistance	Region of resistance	References
Acinetobacter baumannii	Partial +	Pakistan, Europe, Korea	(Antoniadou et al. 2007; Ko et al. 2007; Ahsan et al. 2022)
Pseudomonas aeroginosa	Partial +	Denmark	(Johansen et al. 2008)
Klebsiella pneumonia	Slight +	Pakistan, Thailand, Greece, Italy	(Prevention and Control 2015; Imtiaz et al. 2021; Uzairue et al. 2022)
Proteus mirabilis	Slight +	Lebanon	(Hmede and Kassem 2019)
Serratia marcescens	Partial +	Argentina	(Merkier et al. 2013)
Enterobacter aerogenes	Partial +	Croatia	(Bedenić et al. 2018)
Salmonella enterica	+	USA, Europe, China, Russia	(Elbediwi et al. 2020; Kuleshov et al. 2021; Azizov et al. 2019)
Escherichia coli	Partial +	Pakistan, USA, Europe	(Barlaam et al. 2019; Lv et al. 2018)

A. Intrinsic Resistance Mechanisms

In *P. mirabilis* and *S. marcesens*, resistance to Polymyxins occurs intrinsically by the genetic modification of their LPS. This mechanism is linked by the expression of the *arnBCADTEF* operon and *eptB* gene (Lin et al. 2014). This expression adds 4-amino-4-deoxy-L-arabinose (L-Ara4N) and Phosphoethanolamine (pEtN) to the LPS. It is reported that the LPS of *P. mirabilis* contains L-Ara4N and the genome contains the *eptC* gene (Aquilini et al. 2014). While in *S. marcesens*, *arnB,* and *arnC* mutations lead to resistance. This modification increases the charge on LPS, which decreases Colistin's affinity for LPS (Olaitan, Morand, and Rolain 2014).

B. Acquired Resistance Mechanisms:

i. Chromosomal modification of PmrAB and PhoPQ Two-Component System

Resistance occurs by chromosomal modifications that are like that of bacteria that are naturally resistant to Colistin. The most common mechanism of resistance includes modifications of the LPS membrane via the addition of cationic groups. The *PmrAB* and *PhoPQ* Two-Component System controls the addition of cationic groups to the LPS membrane. The *PmrCAB* Operon encodes 3 proteins i.e. the *pEtN* response regulator *PmrA,* sensor kinase enzyme *PmrAB,* and Phosphotransferase *PmrC*. The *PmrA* and *PmrB* are activated by various environmental stimuli like low pH (5.5), ferric (Fe^{3+}), and Macrophage Phagosome Aluminum (Al^{3+}) (Zhou et al. 2001). Mutations in *PmrA and PmrB genes* lead to Col-R in *K. pneumoniae, Enterobacter aerogenes, and Salmonella Enterica (Gunn 2008).*

ii. CrrAB Two-Component System

It is a Two-Component Regulatory system that changes the *PmrAB* system. The Glycosyltransferase enzyme arises from the mutation in the *CrrB* part of the Two-Component System, which causes modification of the outer LPS membrane. Col-R results from six amino acid substitutions of the Two-Component *CrrB protein*. Mutations of the *CrrB gene* system are caused by overexpression of *CrrC*, this shows that *CrrAB and PmrAB* Two-Component System are indirectly linked via *CrrC*. This causes increased activation of proteins by autophosphorylation which leads to Col-R (Cheng et al. 2016).

iii. Mutation in LPS Synthesis Gene

LPS synthesis is controlled by 3 genes i.e., *ramA, romA,* and *ramR,* where *ramA* and *romA* are regulated by the *ramR* gene. Mutations in these genes cause the modification of the lipid A component of

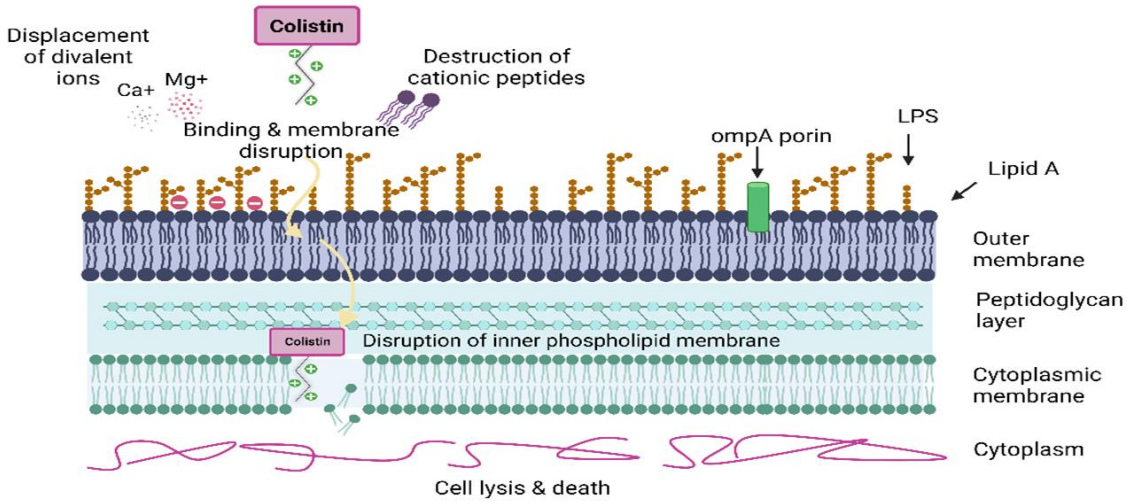

Figure 1 shows colistin binding to lipid A of the bacterial outer membrane, disrupting cationic peptides by displacing divalent ions. In the next step, colistin further disrupts the inner phospholipid membrane by fixing its fatty acid-containing chain/tail, eventually resulting in loss of membrane integrity, cell lysis, and death.

the LPS membrane of GNB. This modification leads to the loss of binding of Colistin to the LPS membrane of bacteria, thereby causing resistance (De Majumdar et al. 2015).

iv. Role of Capsule

In certain bacterial species, the formation of Capsule on the outer surface of bacterial cell wall leads to less affinity of antibiotics to the bacteria (Campos et al. 2004). Capsule acts as a Protective layer against antibiotics in bacteria. The intensity of resistance depends on the number of layers of the Capsule. The more layers, the greater the resistance. Nowadays, Certain bacterial species have formed a capsule around them to become resistant to Colistin (Llobet, Tomas, and Bengoechea 2008).

v. Role of Efflux Pumps

It is seen that *KpnEF, AcrAB, and Sap* protein systems are involved in the activation of efflux pumps in certain bacterial species. A mutation in *KpnEF* leads to increased susceptibility and decreases the MIC of Colistin. The increased activation of Efflux pumps increases the resistance against colistin (Srinivasan and Rajamohan 2013).

vi. Plasmid-Mediated Resistance

Mobile Colistin Resistance (MCR) genes are present in the plasmid genetic material of bacteria. These plasmids are transferred horizontally to other bacteria causing resistance against Colistin to spread. Out of all, the *mcr 1* gene is majorly responsible for the development of resistance against Colistin. *Mcr* genes cause changes in membrane receptors prone to Colistin binding, which in turn increases Col-R (Liu et al. 2016).

5. Detection Methods of Resistance in Colistin

Detection of Col-R is important for managing infections. Susceptibility testing is required to sort out the empirical therapy which is to be followed by the targeted therapy. Challenges have appeared due to the prevalence of colistin-

resistant strains. Handy and rapid methods are required for the diagnosis in laboratories (Kar et al. 2021). Phenotypic and genotypic methods are the cornerstones for determining the prevalent Col-R. They serve as monitoring rules to sway Col-R (Carroll et al. 2019). **Table 2** shows the detection techniques used to detect the resistance against Colistin in bacterial species, along with their principles and brief details.

a. Disk Pre-diffusion Method

The disk diffusion method is a classical method to detect antimicrobial susceptibility. It is based on the principle of diffusion in which the antibiotic from the antibiotic disk diffuses outwards radially and creates a zone of inhibition. This technique is carried out in an agar medium inoculated with bacteria. A paper disk impregnated with the known concentration of colistin is placed on the agar. This method requires 18-24 hours of incubation. (Gounden et al. 2009)

b. Rapid NP Test

Rapid NP Test is an easy and cheap method for the detection of polymyxin resistance and susceptibility. It has a major role in the rapid assortment of empirical treatments based on resistance and susceptibility. AlamarBlue and PrestoBlue also known as resazurin (7-hydroxy-3H-phenoxazin-3 one 10-oxide) are used to grow bacteria. The medium contains a specified concentration of colistin. Bromocresol purple, a pH indicator is used. It changes its color when the medium becomes more alkaline. The color change occurs due to the metabolic activity of the cells which reduces resazurin to resorufin, hence changing the color of the medium to pink. This method detects polymyxin resistance in 3 to 4 hours, which makes it faster than other laboratory techniques. (Bouvier et al. 2021)

c. Inhibition of MCR-1 Activity

Polymixin resistance has been reported to be encountered due to mutations in *the MCR-1 gene.* Phosphoethanolamine transferases are encoded by *MCR genes*. It reduces the negative charge on LPS by adding Phosphoethanolamine to lipid A moiety. It interferes with the binding of colistin to bacterial membranes, eventually causing polymyxin resistance. Zinc is required for *MCR* activity. In this method, polymyxin resistance was decreased by decreasing the concentration of metalloenzyme i.e., Zn by adding EDTA. Another metalloenzyme chelator Dipicolinic acid (DA) is also used for *mcr-1* screening. These scavenge the metal particles which aids in *mcr* activity (Esposito et al. 2017).

d. Loop-Mediated Isothermal Amplification

This is also a robust isothermal technique that requires 30 minutes to 1 hour to amplify nucleic acids. It acquires *Bst Polymerase* which mediates the displacement of DNA strands and DNA auto cycling. This technique involves 4-6 primers which pick out 6-8 attacking regions for amplification in targeted DNA. It is a two-step procedure, in which the first step involves amplification and cycling whereas strand elongation and recycling are performed in the second step (Panno et al. 2020).

e. MIC Strip Colistin

It is a quantitative assay using paper strips to determine minimum inhibitory concentration. The paper strip is impregnated with a defined concentration gradient of colistin. This MIC test strip is placed on an inoculated agar plate. The results are obtained after 18 hours or more of incubation, and the point of intersection of the inhibitory ellipse with the MIC test strip is noticed.

Table 2 shows the detection techniques used to detect Col-R in various strains of Bacteria

Name	Principal	Sample Used	Results	References
Screening of colistin-containing Broth for Polymyxin Resistant Isolates	Broth medium containing Colistin is screened for growth of gram-negative isolates. Daptomycin & Amphotericin B are also added to prevent the growth of Gram-positive bacteria and fungi respectively.	Isolates of GNB (Suspected for polymyxin resistance) are used	Growth of GNB & increased MIC of Polymyxins than usual show resistance to polymyxins	(Nordmann, Jayol, and Poirel 2016b, 2016a)
Disk Pre-diffusion method	By agar dilution method, the MICs & zone of inhibition of Colistin are observed	Samples of bacterial isolates from the gut, mesenteric lymph nodes & feces	MIC> 2µg/ml or smaller zones of inhibition indicates resistance	(Boyen et al. 2010)
Rapid NP Polymyxin test for *Enterobacteriaceae*	The test depends on the pH of the sample. Acid production by bacteria changes the color of the indicator	Blood sample	Color change of indicator within 2 hours.	(Nordmann, Jayol, and Poirel 2016a)
Micromax Assay for *A. baumannii*	Incubation of bacteria in the presence of Colistin	Body fluids containing *A. baumannii* strain	Detection of DNA and cell wall fragments by Fluorescence Microscopy	(Tamayo et al. 2013)
Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF-MS)	Observing the peaks for Lipid A in the membrane of bacteria. Particularly for the detection of *mcr-positive* resistant strains	Isolates of polymyxin-resistant *E. Coli*	Additional peaks at 1919.2 m/z for all polymyxin-resistant strains & 1821 m/z for all *mcr-positive* strains	(Larrouy-Maumus et al. 2016; Dortet et al. 2018)
Inhibition of *MCR-1* Activity	*MCR-1* protein is inhibited either by deprivation of Zn, by addition of Dipicolinic Acid (DPA), or by EDTA	Polymyxin-resistant strains isolated from body fluids	Decrease in MIC of Colistin against resistant strains	(Hinchliffe et al. 2017; Coppi et al. 2018; Esposito et al. 2017)
UMIC Colistine/ MIC Strip Colistin	Test kit containing different conc. Of Colistin to determine susceptibility	Bacterial suspension	Determination of MIC of Colistin against the bacteria	(Bardet et al. 2019)
Loop-Mediated Isothermal Amplification (LAMP)	Amplification of *mcr-1* genes using DNA polymerase with continuous monitoring by assay	Bacterial sample	more sensitive & rapid amplification of *mcr-1* gene than PCR	(Zou et al. 2017)
MicroScan/ Vitek 2	It's an automated system based on fluorometry to detect *mcr-1-tested* isolates	Manually inoculated bacteria on trays	Presence of *mcr-1* positive isolates by fluorometry	(S.Y. Lee et al. 2013)

6. Preventative Measures

Col-R has become a great threat to our healthcare system. It is used as the last resort for drug-resistant infections. Frequent use of colistin, mutation of *mcr* and intrinsic genes and proteins, duration of treatment, and inappropriate dosing and dosing intervals are some of the factors contributing to Col-R. Researchers are still exploring ways to combat Col-R (Sharma et al. 2022). Fluopsin C, a metal-containing antibiotic, and a secondary metabolite, is found to be effective against Gram-positive, Gram-negative, and drug-resistant bacteria. Terrein, another purified metabolite, shows significant antimicrobial activities against *S. aureus, A. hydrophila, E. faecalis*, and other microbes. Therefore, we can suggest that after clinical approval these molecules could be used as potential drugs against colistin-resistant bacteria (Cardozo et al. 2013; Pourhajibagher et al. 2017). Combinatorial therapy is another approach to encountering Col-R. Combination of colistin with Tigecycline (Sheng et al. 2011), Meropenem (C.-H. Lee et al. 2008), Gentamicin, Fosfomycin (Santimaleeworagun et al. 2011), Vancomycin (Hornsey and Wareham 2011), and Rifampicin (Pachón-Ibánez et al. 2010) are reported to be effective against colistin-resistant bacteria. Repurposing drugs is another strategy to overcome this issue. *PFK-158* is an antitumor drug (Pourhajibagher et al. 2017). It has been repurposed against colistin-resistant *Enterobacteriaceae*, for which it showed pharmacodynamic synergism in combination with colistin. Niclosamide is an anthelmintic and non-antibiotic drug

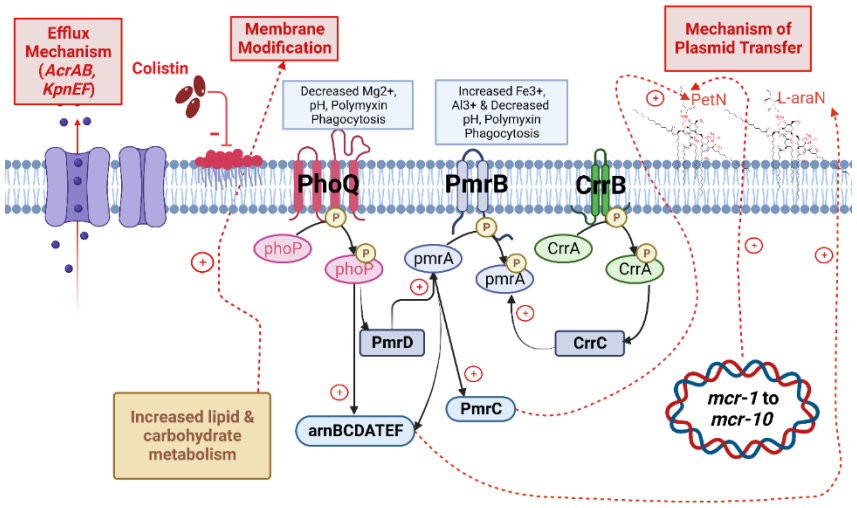

Figure 2 shows resistance mechanisms against Colistin in Gram-Negative Bacteria. (AcrAB, KpnEF activates the efflux mechanisms, modifications in membrane inhibits Colistin binding, PhoPQ, PmrAB, and CrrAB Two-Component Systems inhibits the uptake of Colistin into the bacterial cell and transfer of mcr gene through plasmids which modifies the structure of lipid A in the membrane which inhibits drug binding).

that in combination with colistin can be used against colistin-resistant Gram-negative bacillary infections, and its activity has been assured (Domalaon et al. 2019). Ellipticine and its analogs are reported as an anticancer agent. It is a natural alkaloid. It is a potent molecule against colistin-resistant *E. coli* (Stiborová et al. 2001). AgNPs have bactericidal and inhibitory activity. Conjugation of Colistin and *AgNPs* could enhance the antimicrobial activity and cell biocompatibility. *Col-AgNPs and AgNPs* are biocompatible with human red blood cells and renal cells (Sharma et al. 2022). *Col-AgNPs* have increased activity against GNB i.e., *Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* (Poowadon Muenraya et al. 2022b). *CRISPR/Cas9* approach could be used to delete multiple gene copies by using only one *sgRNA.* It is used to remove plasmids having the *MCR-1* gene in a stepwise manner or simultaneously remove multiple plasmids in one step (P. Wang et al. 2019). Caution should be taken to prevent unwanted recombination events. Combinations of phage *Phab24* with colistin provoked changes in envelope architecture which overcome the resistance in colistin-resistant *Acinetobacter baumannii*. At neutral pH i.e., at pH 7, phage particles were observed to be negatively charged, at the same pH colistin-resistant bacteria had less negative zeta potentials, diminuendo negative surface charge of colistin-resistant cells led to a decrease in the electrostatic repulsion between the bacteria and phage, which promoted phage adherence. This strategy was called Phase Therapy. Photodynamic therapy in combination with colistin showed a high synergetic effect against *A. baumannii*. Expression of the *ompA gene* after PDT in *A. baumannii* helps in more penetration of antibiotics (P. Muenraya et al. 2022a).

Table 3 shows a detailed map for preventative and countermeasures. Moreover, newer drug molecules that are in the pipeline such as QPX7728 which is a cyclic boronic acid analog might be used in combination with colistin in order to counteract the resistance issue. The new drug molecule was found to be effective in multidrug-resistant gram-negative infections when used in combination with other anti-bacterial agents (Hecker et al. 2020; Shareef et al. 2023).

Table 3: Preventive measures used against Colistin resistance

Approach	Molecules/Drugs	Targeted Bacteria/Pathogens	Findings/Effectiveness	References
Metal-Containing Antibiotics	Fluopsin C	Gram-positive, Gram-negative, and drug-resistant bacteria	Effective against colistin-resistant bacteria. Potential future drug after clinical approval.	(Cardozo et al. 2013; Pourhajibagher et al. 2017)
	Terrein	*S. aureus, A. hydrophila, E. faecalis,* and other microbes	Significantly antimicrobial against various bacteria. Potential future drug after clinical approval.	(Cardozo et al. 2013; Pourhajibagher et al. 2017)
Combinatorial Therapy	Colistin + Tigecycline	Colistin-resistant bacteria	Reported effectiveness against colistin-resistant strains.	(Sheng et al. 2011)
	Colistin + Meropenem	Colistin-resistant bacteria	Demonstrated efficacy against colistin-resistant strains.	(C.-H. Lee et al. 2008)
	Colistin + Gentamicin, Fosfomycin	Colistin-resistant bacteria	The combination is reported to be effective against colistin-resistant strains.	(Santimaleeworagun et al. 2011)
	Colistin + Vancomycin	Colistin-resistant bacteria	Reported effectiveness against colistin-resistant strains.	(Hornsey and Wareham 2011)
	Colistin + Rifampicin	Colistin-resistant bacteria	Demonstrated efficacy against colistin-resistant strains.	(Pachón-Ibánez et al. 2010)
Repurposing Drugs	PFK-158	Colistin-resistant Enterobacteriaceae	Repurposed antitumor drug showing synergism with colistin.	(Pourhajibagher et al. 2017)
	Niclosamide	Colistin-resistant Gram-negative bacillary infections	Non-antibiotic drug repurposed in combination with colistin against colistin-resistant infections. Assured activity.	(Domalaon et al. 2019)
	Ellipticine and its analogs	*E. coli*	Natural alkaloid with potent activity against colistin-resistant *E. coli.*	(Stiborová et al. 2001)
Nanoparticle-based Approach	*Col-AgNPs*	GNB (*E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*)	Enhanced antimicrobial activity and biocompatibility. Increased activity against GNB.	(Poowadon Muenraya et al. 2022b)
	AgNPs	Various strains	Bactericidal and inhibitory activity.	[76]
Genetic Approaches	*CRISPR/Cas9*	Deletion of *MCR-1* gene and plasmids containing it	Used for stepwise or simultaneous removal of multiple plasmids with the *MCR-1* gene. Caution is needed to prevent unwanted recombination events.	(P. Wang et al. 2019)
Phage-based Strategies	Phage Phab24 + Colistin	*Acinetobacter baumannii* (colistin-resistant)	Provoked changes in envelope architecture, overcoming resistance. Phase Therapy involves changes in charge promoting phage adherence.	(P. Muenraya et al. 2022a)
	Photodynamic therapy + Colistin	*A. baumannii*	High synergistic effect. *OmpA gene* expression aids antibiotic penetration after photodynamic therapy.	(P. Muenraya et al. 2022a)

7. Challenges and Future Implications

Being a major challenge, Col-R requires multidisciplinary research, appropriate surveillance, and close monitoring to divulge the causes and rate of spread of resistant genes. Colistin binds to Lipid A of LPS and dissolves bacterial outer membrane. Free LPS is released after the death of GNB which is reported to cause endotoxemia (Jansen et al. 2022). Colistin is abducted by the free LPS, which hinders Colistin's activity. To prevent this issue, scientists have been working on creating a supramolecular trap by using subnanometer gold metal sheets (Guo et al. 2022). Lately, it has been found *SAuM* seals off lipid A of free LPS, hence, interceding the interaction between colistin and free LPS. This has helped in increasing the anti-bacterial activity of colistin at low doses which in turn has decreased endotoxin-induced sepsis and bacteremia. Therefore, the supramolecular trap is a possible strategy to circumvent Col-R in the future (F.H. Liao et al. 2020). More robust, rapid, and user-oriented methods are required to determine new resistant bacteria against colistin. These methods can aid in the devising of suitable, less toxic, and potent drugs. Justified use of colistin should be observed to avoid pan-resistant strains in the future.

8. Conclusion

Col-R is an emerging dilemma globally. The injudicious use of colistin in agriculture, animals, aquaculture, and human medicine is accentuating the resistance in GNB. As Colistin is the last resort for gram-negative infections it should be recommended on prescription only, under strict supervision. The bacteria have intrinsic resistance mechanisms which include LPS genetic modification and acquired resistance mechanisms which constitute resistance in *PmrAB* and *PhoPQ*, which helps in acquiring the cationic group in LPS. Other mutations include mutations in the *CrrAB and ramA* genes which also alter the configuration of the LPS membrane of bacteria. Mutation in *KpnEF*, which is involved in the activation of efflux pumps, formation of capsules, and plasma-mediated *mcr* gene resistance is also part of the acquired bacterial resistance mechanism against colistin. Early appropriate therapies are deduced through colistin susceptibility tests, and various antimicrobial susceptibility tests are discussed above, in this study. Furthermore, vigorous, and expeditious detection methods are required to study the resistance patterns clearly and critically in the bacteria treated with colistin. In ongoing epidemiological circumstances, Col-R is being combated with alternative antibiotics and combinatorial therapies which too can pose resistance shortly. Controlled and strict use of colistin might help in overcoming the prevalent Col-R. The findings of the articles may act as a surrogate for devising better ways in AMS programs to reduce the emergence of AMR against colistin.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

NA

Study Approval

NA

Consent Forms

NA

Data Availability

All the raw data related to this study is available with the authors.

Authors' Contribution

MUS designed the study, and MUS, US, and LB wrote the manuscript. US and MUS proofread the manuscript. All authors read and approved the final version of the manuscript.

References

Ahsan, Umaira, Fizza Mushtaq, Sidrah Saleem, Abdul Malik, Hira Sarfaraz, Muhammad Shahzad, Bernt Eric Uhlin, and Irfan Ahmad. 2022. "Emergence of high colistin resistance in carbapenem resistant Acinetobacter baumannii in Pakistan and its potential management through immunomodulatory effect of an extract from Saussurea lappa." *Frontiers in pharmacology* 13: 986802.

Antoniadou, Anastasia, Flora Kontopidou, Garifalia Poulakou, Evangelos Koratzanis, Irene Galani, Evangelos Papadomichelakis, Petros Kopterides, Maria Souli, Apostolos Armaganidis, and Helen Giamarellou. 2007. "Colistin-resistant isolates of Klebsiella pneumoniae emerging in intensive care unit patients: first report of a multiclonal cluster." *Journal of Antimicrobial Chemotherapy* 59 (4): 786-790.

Aquilini, Eleonora, Susana Merino, Yuriy A Knirel, Miguel Regué, and Juan M Tomás. 2014. "Functional identification of Proteus mirabilis eptC gene encoding a core lipopolysaccharide phosphoethanolamine transferase." *International Journal of Molecular Sciences* 15 (4): 6689-6702.

Azizov, Ilya, Eugene Sheck, Marina Sukhorukova, and MV Edelstein. 2019. "Plasmid-mediated resistance to colistin in clinical isolates of Klebsiella spp. and Escherichia coli: results of large retrospective surveillance in Russia." *ECCMID P1413*.

Bardet, Lucie, Liliane Okdah, Stéphanie Le Page, Sophie Alexandra Baron, and Jean-Marc Rolain. 2019. "Comparative evaluation of the UMIC Colistine kit to assess MIC of colistin of gram-negative rods." *BMC microbiology* 19 (1): 1-11.

Barlaam, Alessandra, Antonio Parisi, Elisa Spinelli, Marta Caruso, Pietro Di Taranto, and Giovanni Normanno. 2019. "Global emergence of colistin-resistant Escherichia coli in food chains and associated food safety implications: a review." *Journal of food protection* 82 (8): 1440-1448.

Bashir, Tuba, and Altaf Ahmed. 2016. "Colistin resistance among gram negative organisms; an evolving problem from tertiary care hospital, Pakistan 2014." *American Journal of Microbiology*.

Bedenić, Branka, Mirna Vranić-Ladavac, Carolina Venditti, Arjana Tambić-Andrašević, Nada Barišić, Marija Gužvinec, Natalie Karčić, Nicola Petrosillo, Ranko Ladavac, and Antonino di Caro. 2018. "Emergence of colistin resistance in Enterobacter aerogenes from Croatia." *Journal of Chemotherapy* 30 (2): 120-123.

Bialvaei, A. Z., and H. Samadi Kafil. 2015a. "Colistin, mechanisms and prevalence of resistance." *Curr Med*

Res Opin 31 (4): 707-21. https://doi.org/10.1185/03007995.2015.1018989.

Bialvaei, Abed Zahedi, and Hossein Samadi Kafil. 2015b. "Colistin, mechanisms and prevalence of resistance." *Current medical research and opinion* 31 (4): 707-721.

Biswas, Silpak, Jean-Michel Brunel, Jean-Christophe Dubus, Martine Reynaud-Gaubert, and Jean-Marc Rolain. 2012. "Colistin: an update on the antibiotic of the 21st century." *Expert review of anti-infective therapy* 10 (8): 917-934.

Bouvier, Maxime, Mustafa Sadek, Stefano Pomponio, Fernando D'Emidio, Laurent Poirel, and Patrice Nordmann. 2021. "RapidResa Polymyxin Acinetobacter NP® Test for Rapid Detection of Polymyxin Resistance in Acinetobacter baumannii." *Antibiotics* 10 (5): 558.

Boyen, Filip, Frederic Vangroenweghe, Patrick Butaye, Evelyne De Graef, Frans Castryck, Paul Heylen, Mia Vanrobaeys, and Freddy Haesebrouck. 2010. "Disk prediffusion is a reliable method for testing colistin susceptibility in porcine E. coli strains." *Veterinary microbiology* 144 (3-4): 359-362.

Cai, Yun, Dong Chai, Rui Wang, Beibei Liang, and Nan Bai. 2012. "Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies." *Journal of Antimicrobial Chemotherapy* 67 (7): 1607-1615. https://doi.org/10.1093/jac/dks084. https://doi.org/10.1093/jac/dks084.

Campos, Miguel A, Miguel A Vargas, Verónica Regueiro, Catalina M Llompart, Sebastián Albertí, and José A Bengoechea. 2004. "Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides." *Infection and immunity* 72 (12): 7107-7114.

Cannatelli, Antonio, Tommaso Giani, Marco Maria D'Andrea, Vincenzo Di Pilato, Fabio Arena, Viola Conte, Kyriaki Tryfinopoulou, Alkiviadis Vatopoulos, and Gian Maria Rossolini. 2014. "MgrB inactivation is a common mechanism of colistin resistance in KPC-producing Klebsiella pneumoniae of clinical origin." *Antimicrobial agents and chemotherapy* 58 (10): 5696-5703.

Cardozo, Viviane F, Admilton G Oliveira, Erick K Nishio, Marcia RE Perugini, Célia GTJ Andrade, Wanderley D Silveira, Nelson Durán, Galdino Andrade, Renata KT Kobayashi, and Gerson Nakazato. 2013. "Antibacterial activity of extracellular compounds produced by a Pseudomonas strain against methicillin-resistant Staphylococcus aureus (MRSA) strains." *Annals of clinical microbiology and antimicrobials* 12 (1): 1-8.

Carroll, Laura M, Ahmed Gaballa, Claudia Guldimann, Genevieve Sullivan, Lory O Henderson, and Martin Wiedmann. 2019. "Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible Salmonella enterica serotype Typhimurium isolate." *MBio* 10 (3): 10.1128/mbio. 00853-19.

Cheah, Soon-Ee, Matthew D Johnson, Yan Zhu, Brian T Tsuji, Alan Forrest, Jurgen B Bulitta, John D Boyce, Roger

L Nation, and Jian Li. 2016. "Polymyxin resistance in Acinetobacter baumannii: genetic mutations and transcriptomic changes in response to clinically relevant dosage regimens." *Scientific reports* 6 (1): 26233.

Cheng, Yi-Hsiang, Tzu-Lung Lin, Yi-Tsung Lin, and Jin-Town Wang. 2016. "Amino acid substitutions of CrrB responsible for resistance to colistin through CrrC in Klebsiella pneumoniae." *Antimicrobial agents and chemotherapy* 60 (6): 3709-3716.

Coppi, Marco, Antonio Cannatelli, Alberto Antonelli, Ilaria Baccani, Vincenzo Di Pilato, Samanta Sennati, Tommaso Giani, and Gian Maria Rossolini. 2018. "A simple phenotypic method for screening of MCR-1-mediated colistin resistance." *Clinical Microbiology and Infection* 24 (2): 201. e1-201. e3.

Cortez, Karoll J., Emmanuel Roilides, Flavio Quiroz-Telles, Joseph Meletiadis, Charalampos Antachopoulos, Tena Knudsen, Wendy Buchanan, Jeffrey Milanovich, Deanna A. Sutton, Annette Fothergill, Michael G. Rinaldi, Yvonne R. Shea, Theoklis Zaoutis, Shyam Kottilil, and Thomas J. Walsh. 2008. "Infections Caused by Scedosporium spp." *Clinical Microbiology Reviews* 21 (1): 157-197. https://doi.org/doi:10.1128/cmr.00039-07. https://journals.asm.org/doi/abs/10.1128/cmr.00039-07.

Dadgostar, Porooshat. 2019. "Antimicrobial resistance: implications and costs." *Infection and drug resistance*: 3903-3910.

De Majumdar, Shyamasree, Jing Yu, Maria Fookes, Sean P McAteer, Enrique Llobet, Sarah Finn, Shaun Spence, Avril Monaghan, Adrien Kissenpfennig, and Rebecca J Ingram. 2015. "Elucidation of the RamA regulon in Klebsiella pneumoniae reveals a role in LPS regulation." *PLoS pathogens* 11 (1): e1004627.

Domalaon, Ronald, Oreofe Okunnu, George G Zhanel, and Frank Schweizer. 2019. "Synergistic combinations of anthelmintic salicylanilides oxyclozanide, rafoxanide, and closantel with colistin eradicates multidrug-resistant colistin-resistant Gram-negative bacilli." *The Journal of Antibiotics* 72 (8): 605-616.

Dortet, Laurent, Remy A Bonnin, Ivana Pennisi, Lauraine Gauthier, Agnes B Jousset, Laura Dabos, R Christopher D Furniss, Despoina AI Mavridou, Pierre Bogaerts, and Youri Glupczynski. 2018. "Rapid detection and discrimination of chromosome- and MCR-plasmid-mediated resistance to polymyxins by MALDI-TOF MS in Escherichia coli: the MALDIxin test." *Journal of Antimicrobial Chemotherapy* 73 (12): 3359-3367.

El-Sayed Ahmed, Mohamed Abd El-Gawad, Lan-Lan Zhong, Cong Shen, Yongqiang Yang, Yohei Doi, and Guo-Bao Tian. 2020. "Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019)." *Emerging microbes & infections* 9 (1): 868-885.

Elbediwi, Mohammed, Hang Pan, Silpak Biswas, Yan Li, and Min Yue. 2020. "Emerging colistin resistance in

Salmonella enterica serovar Newport isolates from human infections." *Emerging Microbes & Infections* 9 (1): 535-538.

Esposito, Fernanda, Miriam R Fernandes, Ralf Lopes, Maria Muñoz, Caetano P Sabino, Marcos P Cunha, Ketrin C Silva, Rodrigo Cayô, Willames MBS Martins, and Andrea M Moreno. 2017. "Detection of colistin-resistant MCR-1-positive Escherichia coli by use of assays based on inhibition by EDTA and zeta potential." *Journal of Clinical Microbiology* 55 (12): 3454-3465.

Gounden, Ronald, Colleen Bamford, Richard van Zyl-Smit, Karen Cohen, and Gary Maartens. 2009. "Safety and effectiveness of colistin compared with tobramycin for multi-drug resistant Acinetobacter baumannii infections." *BMC infectious diseases* 9 (1): 1-6.

Gunn, John S. 2008. "The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more." *Trends in microbiology* 16 (6): 284-290.

Guo, Shuwen, Yuling He, Yuanyuan Zhu, Yanli Tang, and Bingran Yu. 2022. "Combatting Antibiotic Resistance Using Supramolecular Assemblies." *Pharmaceuticals* 15 (7): 804.

Hecker, Scott J, K Raja Reddy, Olga Lomovskaya, David C Griffith, Debora Rubio-Aparicio, Kirk Nelson, Ruslan Tsivkovski, Dongxu Sun, Mojgan Sabet, and Ziad Tarazi. 2020. "Discovery of cyclic boronic acid QPX7728, an ultrabroad-spectrum inhibitor of serine and metallo-β-lactamases." *Journal of medicinal chemistry* 63 (14): 7491-7507.

Hinchliffe, Philip, Qiu E Yang, Edward Portal, Tom Young, Hui Li, Catherine L Tooke, Maria J Carvalho, Neil G Paterson, Jürgen Brem, and Pannika R Niumsup. 2017. "Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1." *Scientific reports* 7 (1): 39392.

Hmede, Zaynab, and Issmat I Kassem. 2019. "First report of the plasmid-borne colistin resistance gene (mcr-1) in Proteus mirabilis isolated from a toddler in non-clinical settings." *IDCases* 18: e00651.

Hood, M Indriati, Kyle W Becker, Christelle M Roux, Paul M Dunman, and Eric P Skaar. 2013. "Genetic determinants of intrinsic colistin tolerance in Acinetobacter baumannii." *Infection and immunity* 81 (2): 542-551.

Hornsey, M, and DW Wareham. 2011. "In vivo efficacy of glycopeptide-colistin combination therapies in a Galleria mellonella model of Acinetobacter baumannii infection." *Antimicrobial agents and chemotherapy* 55 (7): 3534-3537.

Imberti, Roberto, Giorgio Antonio Iotti, and Mario Regazzi. 2014. "Intraventricular or intrathecal colistin for the treatment of central nervous system infections caused by multidrug-resistant Gram-negative bacteria." *Expert Review of Anti-infective Therapy* 12 (4): 471-478.

Imtiaz, Wajiha, Zainab Syed, Zara Rafaque, Simon Colin Andrews, and Javid Iqbal Dasti. 2021. "Analysis of antibiotic resistance and virulence traits (genetic and phenotypic) in Klebsiella pneumoniae clinical

isolates from Pakistan: identification of significant levels of carbapenem and colistin resistance." *Infection and drug resistance*: 227-236.

Jansen, Wiebke, Jobke van Hout, Jeanine Wiegel, Despoina Iatridou, Ilias Chantziaras, and Nancy De Briyne. 2022. "Colistin Use in European Livestock: Veterinary Field Data on Trends and Perspectives for Further Reduction." *Veterinary Sciences* 9 (11): 650.

Jeannot, Katy, Arnaud Bolard, and Patrick Plesiat. 2017. "Resistance to polymyxins in Gram-negative organisms." *International journal of antimicrobial agents* 49 (5): 526-535.

Johansen, Helle Krogh, Samuel M Moskowitz, Oana Ciofu, Tacjana Pressler, and Niels Høiby. 2008. "Spread of colistin resistant non-mucoid Pseudomonas aeruginosa among chronically infected Danish cystic fibrosis patients." *Journal of Cystic Fibrosis* 7 (5): 391-397.

Kar, P., B. Behera, S. Mohanty, J. Jena, and A. Mahapatra. 2021. "Detection of Colistin Resistance in Carbapenem Resistant Enterobacteriaceae by Reference Broth Microdilution and Comparative Evaluation of Three Other Methods." *J Lab Physicians* 13 (3): 263-269. https://doi.org/10.1055/s-0041-1731137.

Ko, Kwan Soo, Ji Yoeun Suh, Ki Tae Kwon, Sook-In Jung, Kyong-Hwa Park, Cheol In Kang, Doo Ryeon Chung, Kyong Ran Peck, and Jae-Hoon Song. 2007. "High rates of resistance to colistin and polymyxin B in subgroups of Acinetobacter baumannii isolates from Korea." *Journal of Antimicrobial Chemotherapy* 60 (5): 1163-1167.

Kuleshov, Konstantin V, Anastasia S Pavlova, Elizaveta D Shedko, Yulia V Mikhaylova, Gabriele Margos, Sabrina Hepner, Igor V Chebotar, Elena V Korneenko, Alexander T Podkolzin, and Vasiliy G Akimkin. 2021. "Mobile colistin resistance genetic determinants of non-typhoid salmonella enterica isolates from Russia." *Microorganisms* 9 (12): 2515.

Larrouy-Maumus, Gerald, Abigail Clements, Alain Filloux, Ronan R McCarthy, and Serge Mostowy. 2016. "Direct detection of lipid A on intact Gram-negative bacteria by MALDI-TOF mass spectrometry." *Journal of microbiological methods* 120: 68-71.

Lee, Chen-Hsiang, Ya-Fen Tang, Lin-Hui Su, Chun-Chih Chien, and Jien-Wei Liu. 2008. "Antimicrobial effects of varied combinations of meropenem, sulbactam, and colistin on a multidrug-resistant Acinetobacter baumannii isolate that caused meningitis and bacteremia." *Microbial Drug Resistance* 14 (3): 233-237.

Lee, Sang-Yeop, Sung Ho Yun, Hayoung Lee, Yoon-Sun Yi, Edmond Changkyun Park, Wooyoung Kim, Hye-Yeon Kim, Je Chul Lee, Gun-Hwa Kim, and Seung Il Kim. 2020. "Analysis of the extracellular proteome of colistin-resistant Korean acinetobacter baumannii strains." *ACS omega* 5 (11): 5713-5720.

Lee, Seung Yeob, Jong Hee Shin, Kyungwon Lee, Min Young Joo, Kyung Hwa Park, Myung Geun Shin, Soon Pal Suh, Dong Wook Ryang, and Soo Hyun Kim. 2013. "Comparison of the Vitek 2, MicroScan, and Etest

methods with the agar dilution method in assessing colistin susceptibility of bloodstream isolates of Acinetobacter species from a Korean university hospital." *Journal of clinical microbiology* 51 (6): 1924-1926.

Li, Jian, Roger L Nation, Robert W Milne, John D Turnidge, and Kingsley Coulthard. 2005. "Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria." *International journal of antimicrobial agents* 25 (1): 11-25.

Liao, Fang-Hsuean, Te-Haw Wu, Chun-Nien Yao, Shu-Chen Kuo, Chun-Jen Su, U-Ser Jeng, and Shu-Yi Lin. 2020. "A supramolecular trap to increase the antibacterial activity of colistin." *Angewandte Chemie International Edition* 59 (4): 1430-1434.

Liao, Weichao, Yushan Cui, Jingjing Quan, Dongdong Zhao, Xinhong Han, Qiucheng Shi, Qian Wang, Yan Jiang, Xiaoxing Du, and Xi Li. 2022. "High prevalence of colistin resistance and mcr-9/10 genes in Enterobacter spp. in a tertiary hospital over a decade." *International journal of antimicrobial agents* 59 (5): 106573.

Lima, Tiago, Sara Domingues, and Gabriela Jorge Da Silva. 2019. "Plasmid-mediated colistin resistance in Salmonella enterica: a review." *Microorganisms* 7 (2): 55.

Lima, William Gustavo, Mara Cristina Alves, Waleska Stephanie Cruz, and Magna Cristina Paiva. 2018. "Chromosomally encoded and plasmid-mediated polymyxins resistance in Acinetobacter baumannii: a huge public health threat." *European Journal of Clinical Microbiology & Infectious Diseases* 37: 1009-1019.

Lin, Quei Yen, Yi-Lin Tsai, Ming-Che Liu, Wei-Cheng Lin, Po-Ren Hsueh, and Shwu-Jen Liaw. 2014. "Serratia marcescens arn, a PhoP-regulated locus necessary for polymyxin B resistance." *Antimicrobial agents and chemotherapy* 58 (9): 5181-5190.

Liu, Yi-Yun, Yang Wang, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, and Xianhui Huang. 2016. "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study." *The Lancet infectious diseases* 16 (2): 161-168.

Llobet, Enrique, Juan M Tomas, and Jose A Bengoechea. 2008. "Capsule polysaccharide is a bacterial decoy for antimicrobial peptides." *Microbiology* 154 (12): 3877-3886.

Lv, Jiali, Mashkoor Mohsin, Sheng Lei, Swaminath Srinivas, Raja Talish Wiqar, Jingxia Lin, and Youjun Feng. 2018. "Discovery of a mcr-1-bearing plasmid in commensal colistin-resistant Escherichia coli from healthy broilers in Faisalabad, Pakistan." *Virulence* 9 (1): 994-999.

Mendes, Carlos Alberto Caldeira, and Emmanuel A Burdmann. 2009. "Polymyxins: review with emphasis on nephrotoxicity." *Revista da Associação Médica Brasileira* 55: 752-759.

Merkier, Andrea Karina, María Cecilia Rodríguez, Ana Togneri, Silvina Brengi, Carolina Osuna, Mariana Pichel, Marcelo H Cassini, Serratia

marcescens Argentinean Collaborative Group, and Daniela Centrón. 2013. "Outbreak of a cluster with epidemic behavior due to Serratia marcescens after colistin administration in a hospital setting." *Journal of clinical microbiology* 51 (7): 2295-2302.

Moffatt, Jennifer H, Marina Harper, Paul Harrison, John DF Hale, Evgeny Vinogradov, Torsten Seemann, Rebekah Henry, Bethany Crane, Frank St. Michael, and Andrew D Cox. 2010. "Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production." *Antimicrobial agents and chemotherapy* 54 (12): 4971-4977.

Moghnieh, Rima A, Jihane A Moussa, Mohamed Abdel Aziz, and Ghassan M Matar. 2021. "Phenotypic and genotypic characterisation of cephalosporin-, carbapenem-and colistin-resistant Gram-negative bacterial pathogens in Lebanon, Jordan and Iraq." *Journal of global antimicrobial resistance* 27: 175-199.

Morrison, Lindsay, and Teresa R Zembower. 2020. "Antimicrobial resistance." *Gastrointestinal Endoscopy Clinics* 30 (4): 619-635.

Muenraya, P., S. Sawatdee, T. Srichana, and A. Atipairin. 2022a. "Silver Nanoparticles Conjugated with Colistin Enhanced the Antimicrobial Activity against Gram-Negative Bacteria." *Molecules* 27 (18). https://doi.org/10.3390/molecules27185780.

Muenraya, Poowadon, Somchai Sawatdee, Teerapol Srichana, and Apichart Atipairin. 2022b. "Silver nanoparticles conjugated with colistin enhanced the antimicrobial activity against gram-negative bacteria." *Molecules* 27 (18): 5780.

Muller, Cédric, Patrick Plésiat, and Katy Jeannot. 2011. "A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β-lactams in Pseudomonas aeruginosa." *Antimicrobial agents and chemotherapy* 55 (3): 1211-1221.

Nation, Roger L, and Jian Li. 2009. "Colistin in the 21st century." *Current Opinion in Infectious Diseases* 22 (6): 535-543. https://doi.org/10.1097/QCO.0b013e328332e672. https://journals.lww.com/co-infectiousdiseases/Fulltext/2009/12000/Colistin_in_the_21st_century.4.aspx.

Nordmann, Patrice, Aurélie Jayol, and Laurent Poirel. 2016a. "Rapid detection of polymyxin resistance in Enterobacteriaceae." *Emerging infectious diseases* 22 (6): 1038.

---. 2016b. "A universal culture medium for screening polymyxin-resistant Gram-negative isolates." *Journal of clinical microbiology* 54 (5): 1395-1399.

Olaitan, Abiola O, Serge Morand, and Jean-Marc Rolain. 2014. "Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria." *Frontiers in microbiology* 5: 643.

Pachón-Ibánez, María E, Fernando Docobo-Pérez, Rafael López-Rojas, Juan Domínguez-Herrera, Manuel E Jiménez-Mejias, Andrés García-Curiel, Cristina Pichardo, Luis Jiménez, and Jerónimo Pachón. 2010. "Efficacy of rifampin and its combinations with imipenem,

sulbactam, and colistin in experimental models of infection caused by imipenem-resistant Acinetobacter baumannii." *Antimicrobial agents and chemotherapy* 54 (3): 1165-1172.

Panno, Stefano, Slavica Matić, Antonio Tiberini, Andrea Giovanni Caruso, Patrizia Bella, Livio Torta, Raffaele Stassi, and Salvatore Davino. 2020. "Loop mediated isothermal amplification: principles and applications in plant virology." *Plants* 9 (4): 461.

Petrosillo, Nicola, Fabrizio Taglietti, and Guido Granata. 2019. "Treatment Options for Colistin Resistant Klebsiella pneumoniae: Present and Future." *Journal of Clinical Medicine* 8 (7): 934. https://www.mdpi.com/2077-0383/8/7/934.

Pourhajibagher, Maryam, Hosein Kazemian, Nasim Chiniforush, and Abbas Bahador. 2017. "Evaluation of photodynamic therapy effect along with colistin on pandrug-resistant Acinetobacter baumannii." *Laser Therapy* 26 (2): 97-103.

Prevention, European Centre for Disease, and Control. 2015. "Antimicrobial resistance surveillance in Europe 2015. annual report of the European antimicrobial resistance surveillance network (EARS-Net)." *ECDC*.

Sampson, Timothy R, Xiang Liu, Max R Schroeder, Colleen S Kraft, Eileen M Burd, and David S Weiss. 2012. "Rapid killing of Acinetobacter baumannii by polymyxins is mediated by a hydroxyl radical death pathway." *Antimicrobial agents and chemotherapy* 56 (11): 5642-5649.

Santimaleeworagun, Wichai, Payom Wongpoowarak, Pantip Chayakul, Sutthiporn Pattharachayakul, Pimpimon Tansakul, and Kevin W Garey. 2011. "In vitro activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant Acinetobacter baumannii producing OXA-23 carbapenemases." *Southeast Asian Journal of Tropical Medicineand Public Health* 42 (4): 890.

Septimus, E. J. 2018. "Antimicrobial Resistance: An Antimicrobial/Diagnostic Stewardship and Infection Prevention Approach." *Med Clin North Am* 102 (5): 819-829. https://doi.org/10.1016/j.mcna.2018.04.005.

Shareef, Usman, Aisha Altaf, Madiha Ahmed, Nosheen Akhtar, Muhammad S Almuhayawi, Soad K Al Jaouni, Samy Selim, Mohamed A Abdelgawad, and Mohammed K Nagshabandi. 2023. "A Comprehensive review of Discovery and Development of drugs discovered from 2020-2022." *Saudi Pharmaceutical Journal*: 101913.

Sharma, J., D. Sharma, A. Singh, and K. Sunita. 2022. "Colistin Resistance and Management of Drug Resistant Infections." *Can J Infect Dis Med Microbiol* 2022: 4315030. https://doi.org/10.1155/2022/4315030.

Sheng, Wang-Huei, Jann-Tay Wang, Shu-Ying Li, Yu-Chi Lin, Aristine Cheng, Yee-Chun Chen, and Shan-Chwen Chang. 2011. "Comparative in vitro antimicrobial susceptibilities and synergistic activities of antimicrobial

combinations against carbapenem-resistant Acinetobacter species: Acinetobacter baumannii versus Acinetobacter genospecies 3 and 13TU." *Diagnostic microbiology and infectious disease* 70 (3): 380-386.

Snitkin, Evan S, Adrian M Zelazny, Jyoti Gupta, Tara N Palmore, Patrick R Murray, Julia A Segre, and NISC Comparative Sequencing Program. 2013. "Genomic insights into the fate of colistin resistance and Acinetobacter baumannii during patient treatment." *Genome research* 23 (7): 1155-1162.

Srinivasan, Vijaya Bharathi, and Govindan Rajamohan. 2013. "KpnEF, a new member of the Klebsiella pneumoniae cell envelope stress response regulon, is an SMR-type efflux pump involved in broad-spectrum antimicrobial resistance." *Antimicrobial agents and chemotherapy* 57 (9): 4449-4462.

Stiborová, Marie, Christian A Bieler, Manfred Wiessler, and Eva Frei. 2001. "The anticancer agent ellipticine on activation by cytochrome P450 forms covalent DNA adducts." *Biochemical pharmacology* 62 (12): 1675-1684.

Tamayo, Maria, Rebeca Santiso, Fátima Otero, Germán Bou, José Antonio Lepe, Michael J McConnell, José Miguel Cisneros, Jaime Gosálvez, and José Luis Fernández. 2013. "Rapid determination of colistin resistance in clinical strains of Acinetobacter baumannii by use of the micromax assay." *Journal of Clinical Microbiology* 51 (11): 3675-3682.

Tyson, Gregory H, Cong Li, Chih-Hao Hsu, Sherry Ayers, Stacey Borenstein, Sampa Mukherjee, Thu-Thuy Tran, Patrick F McDermott, and Shaohua Zhao. 2020. "The mcr-9 gene of Salmonella and Escherichia coli is not associated with colistin resistance in the United States." *Antimicrobial Agents and Chemotherapy* 64 (8): 10.1128/aac. 00573-20.

Uzairue, Leonard Ighodalo, Ali A Rabaan, Fumilayo Ajoke Adewumi, Obiageli Jovita Okolie, Jamiu Bello Folorunso, Muhammed A Bakhrebah, Mohammed Garout, Wadha A Alfouzan, Muhammad A Halwani, and Aref A Alamri. 2022. "Global prevalence of colistin resistance in Klebsiella pneumoniae from bloodstream infection: A systematic review and meta-analysis." *Pathogens* 11 (10): 1092.

Vidaillac, Céline, Lothaire Benichou, and Raphaël E Duval. 2012. "In vitro synergy of colistin combinations against colistin-resistant Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella pneumoniae isolates." *Antimicrobial agents and chemotherapy* 56 (9): 4856-4861.

Wang, Pengxia, Dongmei He, Baiyuan Li, Yunxue Guo, Weiquan Wang, Xiongjian Luo, Xuanyu Zhao, and Xiaoxue Wang. 2019. "Eliminating mcr-1-harbouring plasmids in clinical isolates using the CRISPR/Cas9 system." *Journal of Antimicrobial Chemotherapy* 74 (9): 2559-2565.

Wang, Yanan, Fei Liu, Yongfei Hu, Gaiping Zhang, Baoli Zhu, and George Fu Gao. 2020. "Detection of mobile

colistin resistance gene mcr-9 in carbapenem-resistant Klebsiella pneumoniae strains of human origin in Europe." *Journal of Infection* 80 (5): 578-606.

Watkins, Richard R., Tara C. Smith, and Robert A. Bonomo. 2016. "On the path to untreatable infections: colistin use in agriculture and the end of 'last resort' antibiotics." *Expert Review of Anti-infective Therapy* 14 (9): 785-788. https://doi.org/10.1080/14787210.2016.1216314. https://doi.org/10.1080/14787210.2016.1216314.

Zhou, Zhimin, Anthony A Ribeiro, Shanhua Lin, Robert J Cotter, Samuel I Miller, and Christian RH Raetz. 2001. "Lipid A modifications in polymyxin-resistant Salmonella typhimurium: PMRA-dependent 4-amino-4-deoxy-L-arabinose, and phosphoethanolamine incorporation." *Journal of biological chemistry* 276 (46): 43111-43121.

Zou, Dayang, Simo Huang, Hong Lei, Zhan Yang, Yuxin Su, Xiaoming He, Qinghe Zhao, Yong Wang, Wei Liu, and Liuyu Huang. 2017. "Sensitive and rapid detection of the plasmid-encoded colistin-resistance gene mcr-1 in Enterobacteriaceae isolates by loop-mediated isothermal amplification." *Frontiers in microbiology* 8: 2356.