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Colistin the last resort drug in 21st century antibiotics to combat Multidrug resistance superbugs

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ABSTRACT

Polymyxin' E' (Colistin) is considered the last resort therapy against Multidrug resistance (MDR) bacteria, mainly *Klebsiella peumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli* and play a critical role in causing life-threatening infection, and their prevalence is increasing as a big concern globally. Apart from immunological adaptation, chromosomal mutations and plasmid-mediated genes are mostly associated with this resistance at the molecular level. Therefore, the current review extensively focused on Colistin as a drug in 21st-century antibiotics, the activities spectrum with diverse resistance mechanisms of bacteria against Colistin, and emerging approaches of Colistin from discovery to tackling MDR. In the study, we got to know about the challenges and new developments with old weapons like phage therapy as well as new approaches like Phage display and drug repurposing, in addition to the chromosomal and plasmid-mediated genes that play a role in antimicrobial resistance (AMR). The present study would provide insight into the prognostic aspect of combating MDR.

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1 Introduction

Over the last ten years, the prevalence of serious illnesses due to antibiotic-resistant gram-negative bacteria has continued to rise, and these infections now form a serious risk to global public health. Gram-negative bacteria, particularly *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, have caused a massive increase in infection. Several studies have reported polymyxins (A to E) as frequently key accessible active antibiotics agents (Carroll et al. 2019; Lima et al. 2018; Vázquez-López et al. 2020). Where polymyxins E, known as Colistin (Landman et al. 2008; Lima et al. 2018), became accessible for scientific use during the 1960s. However, it was supplanted during the 1970s with different antibiotics inferable from its lethality (Carroll et al. 2019). Use of Colistin was limited when aminoglycosides and other antipseudomonal agents, which were potentially less toxic, became available. Due to increasing reports of nephrotoxicity, intravenous formulations of Polymyxin E and polymyxin B were considerably restricted in practically all countries of the world in the early 1980s (Deris 2015; Deekshit et al. 2023). However, due to MDR (Multiple Drug Resistance), Gram-negative bacteria (GNB) involving patients with cystic fibrosis, the arterial use of Colistin for treating lung infections was routinely limited in the previous two decades (Conway et al. 1997; Mazzitelli et al. 2023). Since then, Polymyxins (Colistin) have been considered a significant therapeutic method capable of acting against Gram-negative bacteria, owing to increasing bacterial resistance to the bulk of widely available antibiotics and a lack of novel drugs. On the other hand, rates of polymyxin E resistance have been comparatively less, likely due to the rare usage; another reason could be the increased formation of colistin-resistant bacteria infection as a result of its use (Zhang et al. 2021; Zhou et al. 2022). Due to the scarcity of novel antibiotics capable of combating GNB, finding a new one will take nine to eleven years (Zhou et al. 2022), so optimizing CMS/colistin is critical.

The increased prevalence of colistin resistance in human and animal species during the past few years can be attributed mainly to the drug's widespread use in livestock and food production. *E. Coli* isolates from swine and bovine sources in Belgium and China harbour variants such as mcr-2 and mcr-3 after the identification of mcr-1 (Timmermans et al. 2021). Moreover, *S. paratyphi* B from German poultry (Borowiak et al. 2019) was found to harbour mcr-5, whereas *Salmonella* spp. and *E. coli* from pigs in Belgium, Italy, and Spain exhibited mcr-4 (Carattoli et al., 2017). Notably, *K. pneumoniae* strains from domesticated animals in China have been found to carry the carbapenemase-encoding gene blaNDM in combination with the discovery of mcr-8 (Mathy et al. 2018). This finding has caused grave concerns because plasmid-mediated mcr-1-associated genes are generally mobile, and colistin resistance may arise quickly in the human microbiome, making colistin therapy ineffective (Zaneveld et al. 2011). Additionally, it was discovered during these surveys that these adjuvants could boost the efficacy of Colistin by more than 2048 folds. This suggests that lower colistin *in-vivo* dosage against Colistin in Gram-negative microorganisms may be possible with adjuvant pairing (Wang et al. 2018; Sheng et al. 2022). This review article investigates the significance of Colistin as a last-resort antibiotic against multidrug-resistant Gram-negative bacteria. This review article also highlighted the colistin efficacy, resistance mechanisms, immunological adaptation and genetic factor development over the last ten years.

2 Colistin

In the subsequent section of this review article, a comprehensive overview of Colistin as a critical antibiotic for combating multidrug-resistant gram-negative bacterial infections and its key features were discussed.

2.1 Colistin 21st century antibiotics overview as a drug

Polymyxin B and Colistin are mostly safe in clinical use. The polymyxins class of antibiotics is dynamic against some selected GNBs, including *P. aeruginosa*, *A. baumannii*, *K. pneumonia*, and *Enterobacter* species (Van Loon et al. 2017). Colistin is the last line of antibiotics treated against multidrug-resistant gram-negative bacterial infection caused by ESKAPE pathogens. Currently, the therapeutic option against carbapenem resistance Gram-negative bacilli creates a great problem in clinical practices. Moreover, the increasing rate of drug resistance is known as 'superbugs' that give new challenges for scientists (Falagas et al. 2005; Giamarellou and Poulakou, 2009; Tan and Tatsumura 2015).

Antibiotic resistance is not only a sectional issue but a worldwide one. Typically, bacteria resist antibiotics, limiting the treatment option, raising mortality and morbidity, and increasing the hazard of antibiotics-related adverse situations. The resistance to antibiotics is termed as the ability of a microorganism to withstand the impacts of antibiotics, and it is a sort of medication opposition. Antibiotic resistance develops using natural selection via random mutation; likewise, it could be built by applying a developmental weight to a population. When such a gene is formed, the microorganisms can transfer the hereditary information horizontally (Between the individuals) by plasmid exchange. If a bacterium transmits a few resistance genes, it is called multidrug resistance or a superbug. So, Colistin has been re-surveyed as a fundamentally significant antimicrobial in people because of its efficiency against multidrug-resistant Gram-negative bacteria, specifically against *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*. In the 21st century, Colistin is probably the last combat antibiotic against gram-negative multidrug-resistant pathogens.

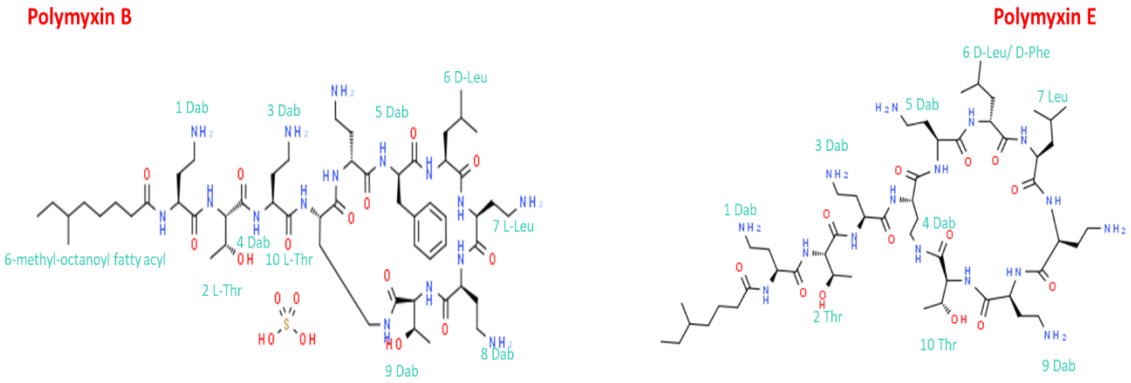

Figure 1 Structure difference between Polymixin B and Polymixin E

The closest look at antibiotic resistance will help researchers develop new antibiotics. Keeping away from the wrong use and abuse of antibiotics would hinder the spread of resistant microbes. As microorganisms dependably advance and can grow increasingly more resistant, new antibiotics are expected to fight against them (Figure 1).

3 Structure and Pharmacodynamics properties of Colistin

Colistin is a broad-spectrum antibiotic with a molecular weight of 1750 Da. Colistin sulfate is cationic, whereas Colistimethatesodium (CMS) is anionic. CMS is modified *in-vivo* to generate polymyxin E, responsible for antibacterial action. A cationic polypeptide rattles the cell membrane through a detergent-like mechanism. Colistimethate sodium is hydrolyzed in aqueous solutions, leading to a composite fusion of sulfomethylated metabolites and Colistin (Michalopoulos and Karatza 2010; Michalopoulos and Falagas 2011; Ayoub Moubareck 2020; Chiu et al. 2022)

4 Mechanism of Action

Polymyxin B and Polymyxin E possess bactericidal activity by binding to lipopolysaccharides (LPS) and phospholipids in Gram-negative microbes' outermost layer (Ayoub Moubareck 2020; Slingerland et al. 2022). Polymyxin-E's Antimicrobial action specifically targets the bacterial cell membrane (Conrad and Galanos 1989). The interaction of cationic polypeptide (Colistin) and anionic lipopolysaccharide (LPS) within the outermost layer of Gram-negative microbes disrupts the cell layer via electrostatic interactions, displacing stabilizing agents such as magnesium (Mg^{2+}) and calcium (Ca2+) from negatively charged LPS particles (Madhumanchi et al. 2020; Tiwari et al. 2022). The Microscopic studies revealed that the bacterial intracellular membrane was partially damaged, releasing cytoplasmic material through membrane gaps (Koike et al. 1969). Because Colistin disrupts membrane integrity, hydrophilic antibiotics such as carbapenem, glycopeptides, rifampicin, and tetracycline have synergistic effects. Although some reports suggest that polymyxins may act through mechanisms other than the bacterial cell layer, the precise mechanism by which Colistin eliminates bacterial cells remains unknown (Ayoub Moubareck 2020).

5 Susceptibility Breakpoint

Polymyxin E susceptibility breakpoints are determined differently in countries, notably Germany, the United Kingdom, and France. Colistin sulphate is commonly used to define sensitivity breakpoints. The French Society for Microbiology has established 2mg/L as the polymyxin E resistance breakpoint, while >2mg/L is generally considered the Enterobacteriaceae resistance breakpoint. On the other hand, the British Society for Antimicrobial Chemotherapy has set the sensitivity and resistance breakpoints at 4mg/L (Andre et al. 2010). Polymyxin E criteria for *Acinetobacter* species (four milligrammes per litre) have been adjusted by the Clinical and Laboratory Standards Institute (CLSI), differing from those specified for *P. aeruginosa* (eight milligrammes per litre) and other non-*Enterobacteriaceae* bacteria (Walsh and Amyes 2004; Karakonstantis 2021). Notably, no polymyxin breakpoints for *Enterobacteriaceae* have been established by CLSI. Colistin disc diffusion testing guidelines have been developed by the French Society of Microbiology and the British Society of Antimicrobial Chemotherapy (Li et al. 2006; Al-Bayssari et al. 2021). However, additional clinical data are required to define optimal susceptibility breakpoints, and standardization across antimicrobial susceptibility testing methods is critical for consistent and reliable results in diverse research settings.

6 Plasmid-mediated colistin resistance gene

Plasmid-mediated polymyxin E resistance emergently has been reported in numerous nations of the globe, including countries like Asia, Africa, Europe, and America (Breazeale et al. 2005; Llobet et al. 2008). The mcr1 gene (mobilized colistin resistance genes) was first identified in human isolates in 2008, and it was highlighted in *Shigella sonnei* from Vietnam (Aghapour et al. 2019). The mcr

gene is particularly concerning since it is present in plasmids, which are very small units of mobile DNA that carry genetic instructions and information from one bacterium to the next (Hassan et al. 2019). This indicates that plasmids carrying resistant mcr genes can transform other bacteria, including carbapenem-resistant *Enterobacteriaceae*, resistant to Colistin (CRE). The term plasmid-mediated polymyxin E resistance (mcr) includes the capability of a gene to survive the effects of Colistin and to spread this ability to other bacteria. These findings suggest that the mcr1 gene has long been present in Enterobacterales but has gone unnoticed (Campos et al. 2004; Biswas et al. 2012). The mcr1 gene has been found in various *Enterobacterales* species, most notably in *E. coli*, *Salmonella*, *Klebsiella*, *Shigella*, *Vibrio*, *Enterobacter*, and others (Dalmolin et al. 2018). Some examples are mcr 1.2, which is isolated from *K. pneumoniae* in Italy; mcr-1.3 and 1.4, which is separated from *E.coli* in China; mcr 1.5, which is isolated from *E.coli* in Argentina, and *Salmonella typhimurium* in China. Further, Mcr 1.7 is isolated from *E. coli* in China, mcr 1.8 from *E.coli* in Brunei and mcr.1.10 is isolated from *Moraxella spp*. in Britain (AbuOun et al. 2018).

Furthermore, mcr-1 exhibits reduced resistance to colistin or polymyxin B, with isolates bearing the mcr-1 gene frequently showing susceptibility to Colistin (Anyanwu et al. 2020). The gene was found to be truncated in an elucidating instance involving a *Shigella sonnei* isolate, elucidating the presence of mcr-1 in isolates susceptible to Colistin (Pham Thanh et al. 2016). Conjugation studies have shown that reactivating the truncated mcr-1 gene can result in the emergence of a colistin-resistant phenotype. The identification of an intact mcr-1 gene in *E. coli* isolates resistant to Colistin, on the other hand, suggests that gene truncation may not be the primary mechanism influencing polymyxin sensitivity in mcr-1-carrying isolates (Liu et al. 2016).

Previously, specific colistin resistance pathways were linked to chromosomal mutations or horizontal gene transfer. Mutations in two-component systems such as PmrA/PmrB and PhoP/PhoQ, as well as changes in the mgrB gene, which encodes a negative regulator of PhoPQ, have been linked to colistin resistance in *K. pneumonia* (Cannatelli et al. 2014). Recent findings, however, indicate the emergence of plasmid-mediated colistin resistance genes in various regions, which contribute significantly to colistin resistance. GenBank currently contains ten distinct sets of mcr genes (mcr-1 to -10) and 18 mcr-1 subtypes (mcr-1.1 to -1.18) (Doumith et al. 2016; Ara et al. 2021) (Figure 2).

The mcr3 had 94.1 % to 94.8 % amino acid sequence similarity with proteins from three Aeromonas species. Similarly, a shortened transposon element (TnAs2), exclusively seen in *Aeromonas salmonicida*, was found upstream of mcr3. These findings imply that mcr3 genes in *Enterobacteraceae* may have originated in the Aeromonas species (Yin et al. 2017). Surprisingly, the mcr4 gene is placed in a tiny, non-self-conjugative plasmid. Including an

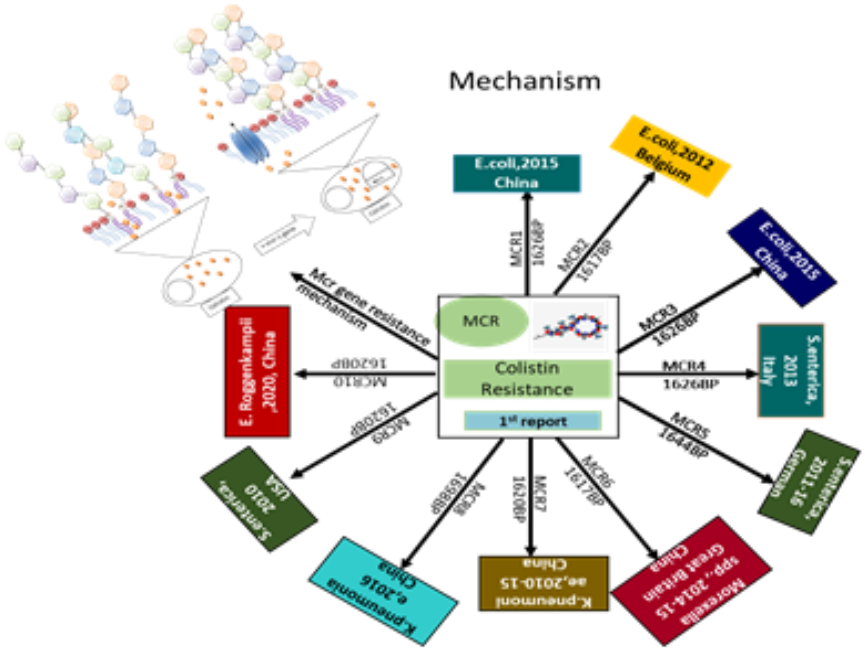

Figure 2 Specific resistance to Colistin is facilitated by the plasmid-mediated mcr-1 gene, encoding a phosphor ethanolamine transferase, which modifies lipid A with a phosphor-ethanolamine (PEP) group, preventing interaction between colistin and lipid A, along with this figure also shows details of mcr-1 to -10 genes

auxiliary plasmid, on the other hand, can increase conjugation. Leaving this variant, there are also reported 13 mcr-1 subgroups throughout the Globes. These type subgroups are only due to differences in only one nucleotide from mcr1. The mcr 1 gene is not limited to the Incl2 plasmid type. It may be found in IncX4, InHI2, IncF, IncHI1, IncY, and IncP plasmid. The MIC for Colistin of the strain with multiple mcr genes was not raised in any scenario (Chambers and Sauer 2013; Srinivasan and Rajamohan 2013) (Figure 2).

7 Phage-mediated colistin resistance

The SCKP83, a *Klebsiella pneumoniae* clinical isolate, has been found to contain colistin-resistant gene mcr-1, making it resistant to Colistin. Zhou et al. (2022) reported an mcr-1-shipping P7 phage-like plasmid of 97.4 kb; out of that, a 90.9-kb region was envisaged to be an integral phage. Even though this plasmid was not self-transmissible, the plasmids that are self-transmissible are found to contain 2600 bp long mcr-1 sequence, flanking at both ends with ISApl1, and there are evident roles of ISApl1 in mcr-1 mobilization (Peirano et al. 2014; Brennan-Krohn et al. 2018). An example of self- transmissible phage like plasmid is pSLK172-1, from a human atypical enteropathogenic *E. coli* that is found to contain phage P1-like sequences along with 12 antimicrobial resistance-encoding genes including florfenicol/chloramphenicol resistance gene (floR), beta-lactams resistance gene (blaCTX-M-14), fosfomycin resistance gene (fosA3) and florfenicol resistance gene (floR), streptomycin resistance (strA), Plasmid-Mediated Sulfamethoxazole resistance (sul2) and tetracycline (tetR).

8 Drug repurposing

Drug repurposing for Colistin is looking for a drug available for treating other diseases but not as antibacterial effects. Various drugs like antibiotics, antimycotics, antihelminth, and other drugs that are currently being used have been evaluated to combat colistin resistance (Figure 3, Table 1). Colistin was initially identified in 1950, and later, it was found to be identical to polymyxin E. Out of the 5 polymyxins (polymyxins A to E), only 2, (polymyxins B and Colistin) are being used in clinical settings. Colistin and polymyxins B differ by only one amino acid (D-phenylalanine to D-leucine) and are used as an alternative to Colistin (Ayerbe-Algaba et al. 2018). However, polymyxin B is moderately effective at clinical concentrations, and the emergence of strains resistant to polymyxin B has been reported. To overcome the resistance, the pharmacokinetic/pharmacodynamic parameters must be checked meticulously, and the average steady-state concentration of the drug should be more or equivalent to the pathogen's MIC to ensure optimal antibacterial action. The grouping of Colistin along with other antibiotics such as azithromycin, linezolid, and rifampin desensitized obtained colistin-resistant bacteria to Colistin, demonstrating that suboptimal concentrations of Colistin along with other antibiotics can produce a synergistic effect to prevent MDR bacteria. Colistin, when combined with antibiotics having protein or RNA synthesis inhibiting roles, poses synergistic effects against colistin-resistant Enterobacteriaceae, and thereby colistin resistance may be prevented by using the drugs in combination (Thangamani et al. 2015; Rajivgandhi et al. 2018) (Figure 3, Table 1).

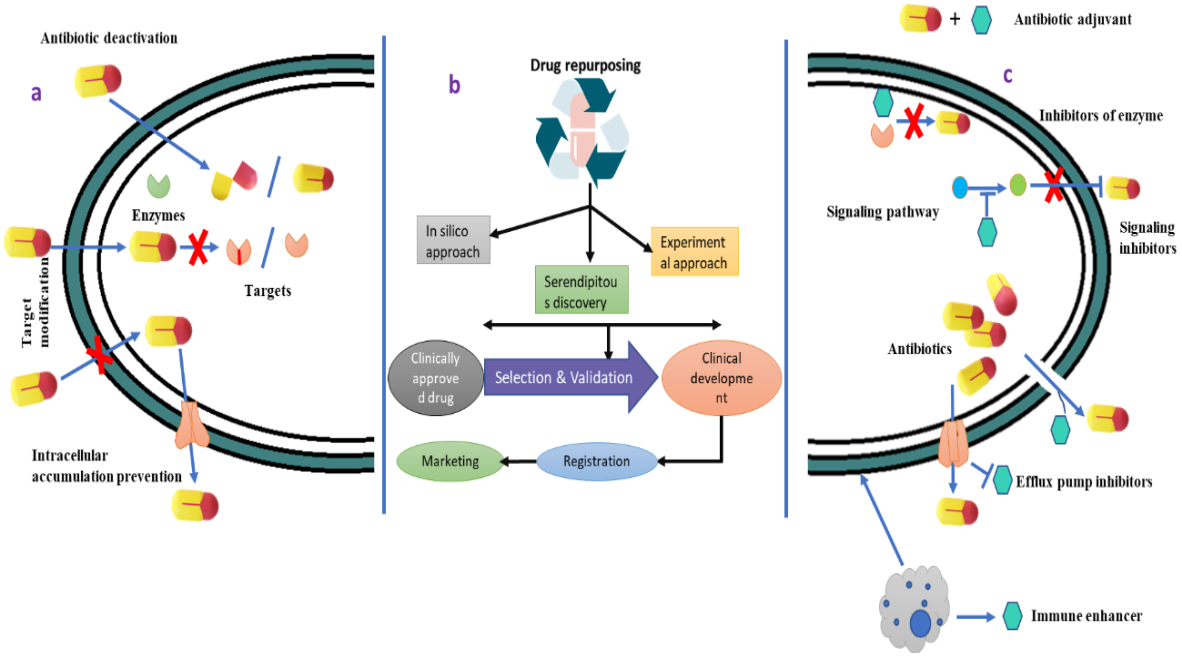

Figure 3 a) Different mechanisms of colistin resistance b) Drug repurposing flowchart c) Combination therapy against AMR

Table 1 The existing drugs of Multidrug resistance bacteria with the mode of action and adverse effects

Groups	Drugs	Mode of action	Bacteria	Important adverse effect	References
Beta-lactams	Penicillins, cephalosporins, carbapenems, monobactams, oxacillin, amoxicillin, amoxyclave, imipenem	Inhibit cell wall synthesis	*Enterobacteriaceae*, *P. aeruginosa*, *Acinetobacter spp*, *Haemophilus influenza*, *E. coli*, *Proteus* spp.	Skin, ENT, UTI, Respiratory infection	Kaur et al. 2011
Glycopeptides	Vancomycins	Inhibit cell wall synthesis	Gram-positive bacteria	MRSA, Skin, Endocarditis	Sarkar et al. 2017
Macrolides & ketolides	Azithromycin, Erythromycin, Clarithromycin	Inhibit protein synthesis (50S)	*S. pneumoniae*, *S. pyogenes*	Pneumoniae, Sinus, ENT, STIs	MacDougall 2017
Aminoglycosides	Gentamycin, Amikacin, Tobramycin, Streptomycin	Inhibit protein synthesis (30S)	*Pseudomonas aeruginosa* and other gram-negative bacilli	Bacteraemia, Abdominal infection	Durante-Mangoni et al. 2009
Tetracyclines	Tetracycline, Tegecycline	Inhibit protein synthesis		Lyme disease, PID, STIs	Abushaheen et al. 2020
Quinolones	Ciprofloxacin, levofloxacin, Moxifloxacin,	Inhibit DNA gyrase	*Campylobacter* spp	Anthrax, Bloodstream infections, Bone infection, Bronchitis, Bladder infection, Bacterial infection, prophylaxis. Campylobacter gastroectasis	Chala and Hamde 2021

9 Nonantibiotic compounds

Various nonantibiotic compounds have been reported to enhance the activity of polymyxins. In this case, antidepressants like amitriptyline, citalopram, imipramine and sertraline worked synergistically with polymyxin B to tackle the infectious bacteria. Similarly, Liu et al. (2023) also report antipsychotics belonging to the phenothiazine family and diuretics like spironolactone and statins (atorvastatin and simvastatin) have been found to act synergistically with polymyxin B to work against multiple drug bacterial infection.

10 Antiviral drugs

The antiviral drug zidovudine is found to be active against HIV (human immunodeficiency virus) and is a nucleoside reverse transcriptase inhibitor. It was the first commercial anti-retroviral for HIV therapy in 1987 (Peyclit et al. 2018). Colistin-resistant *Enterobacteriaceae* members were found to be susceptible to zidovudine with a MIC range between 0.2 – 6.25 μM (0.05 – 1.67 μg/mL). The zidovudine also influenced a clinical isolate of *K. pneumoniae* strain 853. Zidovudine is given at a dose of 600 mg/day for the treatment of HIV, indicating that the plasma concentration of a drug above the MIC can be easily achieved (Chow et al. 2009; Poirel et al. 2016).

11 Anthelmintic Drug

FDA-approved anthelmintic drug Niclosamide acts in synergy with Colistin to eliminate both the Colistin-susceptible and colistin-resistant Gram-negative bacteria isolates. Niclosamide alone has demonstrated a feeble action with a MIC of 512 μg/ml in opposition to the wild-type *P. aeruginosa* PAO1 strain. The MIC is reduced drastically to 2 μg/ml in opposition to efflux deficient PAO750 strain, while MIC remained unchanged in MexAB-OprM tripartite efflux lacking *P. aeruginosa* strain PAO200, and this strongly suggests that the niclosamide is a drug of efflux system (Abavisani et al. 2021). The potentiating effect of niclosamide with Colistin is concentrations above 1 μg/ml (3 μM). The combination of Colistin with Niclosamide has been found effective against both the Colistin susceptible (Col-S) and Colistin resistant (Col-R) *A. baumannii* and *K. pneumonia* (Snesrud et al. 2016).

12 Antineoplastic Drug

A bioactive compound has been isolated from marine endophytic actinomycete *Streptomyces coeruleorubidus* GRG 4 (KY457708) residing in marine macroalgae *Turbinaria ornate*. The active component has anticancer activity. Apart from anticancer activity, it was effective against colistin-resistant uropathogens *P. aeruginosa* and *K. pneumonia* (Bai et al. 2017).

13 Possible future strategic

AMR is the biggest mystery to solve around the globe. The review expands on infectivity due to carbapenem resistance microbes being hard to treat because of the restricted accessibility of therapeutic agents (Giannella et al. 2023). Colistin is commonly

favoured for treating cases brought about by pan-drug-resistant strains of carbapenemase producers despite its toxicity. Other than Colistin, different other anti-microbials in the polymyxin groups are dynamic against those Gram-negative microbes that include *Klebsiella* species, *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Enterobacter* species. Colistin is a viable suitable antimicrobial agent for treating a wide range of multidrug-resistant Gram-negative microbes. It is a last-line drug to fight against serious diseases caused by gram-negative microorganisms (Bialvaei and SamadiKafil 2015; Liu et al. 2023). Different therapeutic management is considered to overcome the administration of last-line antibiotics. During the most recent couple of years, it's also been observed from Japan in science news, which was published in the Journal of Antimicrobial Chemotherapy, that nightmare bacteria exhibiting resistance to Colistin, which was the last resort therapy against MDR (Giurazza et al. 2021). Colistin obstruction is a significant issue because of the absence of particular antibiotics. Many known methods of investigation for AMR have fizzled, so we have to return to the planning phase and search for new ones. One promising approach is using bioinformatics in blend with systems biology and synthetic biology to recognize and deliver novel antibiotics through mining genome and metagenome sequence information for BGCs (biosynthetic gene clusters) (Navid et al. 2009). BGC generally encodes antimicrobial molecules, like non-ribosomal peptides, polypeptide antibiotics, terpenoids, alkaloids, saccharides and bacteriocins, which mostly regulate pathogenic microorganisms.

Two approaches that can create more impact in this multiple drug resistance bacterial infection management are systems biology and synthetic biology. Systems biology signifies creating novel processes to consider the usefulness of the living system as an entire (Yan et al. 2023). When contemplating bacteria, these approaches assist in comprehending how microbes evolve, acclimatize, and interrelate with other living beings. It also uncovers the outline and the dynamics of metabolites, proteins, and RNAs. It also deciphers their intracellular interactions and reveals the complex regulatory networks. Another strategy is synthetic biology, which creates artificial implements to achieve specific functions. Microbes are excellent hosts for significant purposes, such as bioconversion, bioproduction, biodegradation, and bioremediation. Predominantly, the engineered microorganisms have been broadly employed to make therapeutic proteins, chemicals, enzymes, biofuels, small molecular pharmaceuticals and other materials. Despite all these facts, the systems and synthetic biology focus on research and innovation. It carries out the goal of better-engineered research tools, which can then provide experiences to systems biology. In this unique circumstance, drug repurposing, which consists of utilizing a nonantibiotic compound to treat MDR, is supported. Using novel antibiotics like beta-lactam/beta-lactamase inhibitor-based and non-beta lactam-based agents could greatly relieve. To prevent the spread of disease and oncoming septicemia, an effective drug routine is an essential requirement. So, a treatment strategy must be commenced considering all these considerations.

Conclusion

This comprehensive review elucidates the significance of Colistin in the fight against MDR bacteria, highlights the challenges posed by resistance mechanisms, and suggests potential future directions. Combating the looming threat of antimicrobial resistance requires a multifaceted approach that includes drug repurposing, innovative biological methodologies, and robust surveillance systems.

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Declarations

No declaration.

Conflict of interest

The authors declare no conflicts of interest.

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