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Synergistic Approaches to Combatting Polymyxin- Resistant Microbial Strains in Hospital Settings: Diagnostics, Clinical Outcomes, Policy Implications and Current Challenges in Treating

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Abstracts

Polymyxins include polymyxin B and colistin, antibiotics which have played a crucial role as last resorts in the treatment of drug-resistant Gram-negative bacterial infections. Nevertheless, the increasing emergence of strains resistant to polymyxins has posed considerable clinical treatment challenges that risk the world's public health. Such resistance mechanisms include

lipid A modification, efflux pumps, and plasmid-mediated mcr genes. These mechanisms render the polymyxins less potent, thus complicating infection control in clinical settings, especially hospitals. The present strategies against resistance are combination therapies: polymyxin with β-lactams, EDTA adjuvants, non-antibiotic approaches-nanoparticles, and phage therapy. Better diagnostics together with antimicrobial stewardship programs will control the spreading of resistance. MALDI-TOF and Rapid Polymyxin NP test can rapidly diagnose the diseases that can treat quickly. The development of solutions like host-based therapies, immunotherapy, and safer polymyxin analogues will be useful to cope with this crucial challenge. It pays more attention toward research and development of the novel therapy for polymyxin-resistant infections by providing much emphasis on global cooperation as well as government policies on combating the problem of antimicrobial resistance.

Keywords: Polymyxin resistance, Gram-negative bacteria, Combination therapy, Antimicrobial stewardship, Lipid A modification, Rapid diagnostics, Immunotherapy.

1. Introduction

Last-resort medicines called polymyxins are used to treat Gram-negative bacterial infections that are resistant to many drugs. (Yu et al., 2015). They belong to the polymyxins A-E and among these, there are two clinically used forms known as Colistin, commonly known as polymyxin B and polymyxin E, (Yu et al., 2015). The breakdown of the bacterial wall's outer membrane is the main way that polymyxins exert their antibacterial activity (Tran et al., 2015). The negatively charged phosphate groups of the lipid are electrostatically interacting with the cationic L-α, γ-diaminobutyric acid (Dab) side chains of polymyxins. One of the main constituents of the Gram-negative outer membrane is lipopolysaccharide (LPS) (Tran et al., 2015). This interaction displaces the divalent cations (Ca^{2+} and Mg^{2+}) that typically bridge neighboring LPS molecules, resulting to disarray and increased permeability of the outer membrane (Tran et al., 2015). Although the exact mechanisms of polymyxin-mediated killing of bacteria are not well understood, it is thought that this outer membrane disruption is an important step (Tran et al., 2015).

There are several reasons why polymyxins are last-resort antibiotics. First, there is an emerging major problem of polymyxin resistance primarily due to mechanisms that involve specific modifications of the lipid A component of LPS (Qi et al., 2022; Moffatt et al., 2010). Even when this happens, the resistance occurs in the absence of previous exposure to polymyxin (Xu et al., 2022). Second, the therapeutic index of polymyxins is narrow and they possess strong nephrotoxic and neurotoxic potentials; hence, their usage has been limited (Yu et al., 2015; Oliveira et al., 2020). Thirdly, the polymyxin class has been abandoned for more than half a century, especially after the discovery of less toxic antibiotics, which limited further drug development in the said class (Yu et al., 2015; Oliveira et al., 2020).

The alarming rise in carbapenem-resistant Gram-negative infections has pushed the resurgence of polymyxins as a treatment option since these may be the last effective antibiotic for such infections (Perez et al., 2020). However, polymyxins have rapidly become the true last line of protection from MDR Gram-negative bacteria, often accompanied by high mortality rates (Oliveira et al., 2020; Xu et al., 2022). Polymyxin-resistant microbial strains are highly

dangerous to the clinical environment as they can easily develop resistance against one of the last drugs available in the treatment of multidrug-resistant Gram-negative bacterial infections, Maifiah et al., 2016; Qian et al., 2012; Velkov et al., 2014. These strains developed resistance to the antimicrobial effects of polymyxins. These antibiotics target the cell membrane of bacteria (Arroyo et al., 2011;, Tsuji et al., 2016).

One of the critical resistance mechanisms to polymyxin is the alteration of the lipid A moiety of the bacterial lipopolysaccharide (LPS) (Arroyo et al., 2011;, Moffatt et al., 2010).This changes in the form of added positive charges, such as phosphoethanolamine or 4-amino-4-deoxy-L-arabinose leads to a decreased binding of the polymyxins to the cell membrane of the bacteria, (Arroyo et al., 2011;, Moffatt et al., 2010). On the other hand, some polymyxin-resistant bacteria also lack LPS completely, such as in the case of Acinetobacter baumannii, which limits the use of the polymyxin. This third mechanism of polymyxin resistance is through the expression of efflux pumps at highly elevated levels. This pump expels the antibiotic outside the bacterial cell. Some two months ago, it has been shown that an efflux pump gene on a genomic island bestows resistance to polymyxin in Pandoraea pnomenusa (Gao et al., 2023).

These polymyxin-resistant strains raise great concern in hospitals where these organisms cause serious and hard-to-treat infections (Zimmerman et al., 2020; Xiao et al., 2023). Polymyxins and colistin and polymyxin B are drugs used only to treat resistant Gram-negative bacteria infections, most of which include Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Acinetobacter baumannii, as reported by Qian et al., 2012; Xiao et al., 2023, where it has been noted that 2.6% resistant strains dominate the hospitals. and other health care set-ups of the nation (Xiao et al., 2023). These resistant strains normally tend to become cross-resistant against other drugs like carbapenems, so their use is now becoming difficult (Xiao et al., 2023). Further, using polymyxins has led to the development of resistance, an issue with the underlying fact about stewardship with research and developments on new antimicrobial measures (Tsuji et al., 2016).

2. Understanding Polymyxin Resistance

The primary mechanisms include modifications of the lipid A component of the bacterial outer membrane (Han et al., 2017; Velkov et al., 2014; Deris et al., 2014). Gram-negative bacteria achieve this by introducing cationic modifications, such as adding the L-Ara4N (4-amino-4-deoxy-L-arabinose) or pEtN (phosphoethanolamine) to lipid A phosphate groups (Velkov et al., 2014; Deris et al., 2014; Han et al., 2017). This will repel the electrostatic attraction of the outer membrane from the positively charged polymyxin molecules, thus decreasing the net negative charge of the outer membrane (Han et al., 2017; Velkov et al., 2014; Deris et al., 2014).

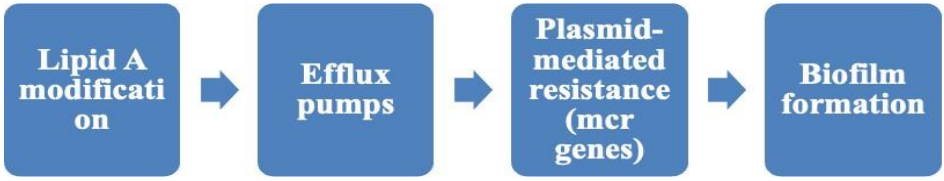

Figure 1. Resistance Mechanisms of Polymyxins

The upregulation of the pmrAB two-component system and the arnBCDTEF-ugd operon, which add L-Ara4N to lipid A, has been reported in polymyxin-susceptible Pseudomonas aeruginosa strains when exposed to polymyxin B (Han et al., 2017). Furthermore, the deletion of the pagL gene, which is involved in lipid A deacylation, significantly increased the polymyxin susceptibility of the polymyxin-resistant P. aeruginosa strain (Han et al., 2017). These findings indicate that L-Ara4N-modified lipid A is the most critical resistance mechanism of polymyxins in P. aeruginosa (Han et al., 2017). Another mechanism in A. baumannii by pmrCAB operon leads to resistance through modifications of the lipid A by the addition of phosphoethanolamine to the molecule (Arroyo et al., 2011). This reduces the net negative charge of the outer membrane and helps prevent the permeabilizing activity of polymyxins.

Another mechanism of resistance by polymyxin involves the action of efflux pumps. Although the role of efflux pumps in resisting polymyxin has not been studied in detail in these references, it is established that the action of efflux systems does allow for the extrusion of antimicrobial agents, like the polymyxins, out of the bacterial cell (Zimmerman et al., 2020). These other concerns also include increased plasmid-mediated polymyxin resistance, which includes resistance conferred by the mcr genes (Hussein et al., 2018; Vieira, 2024; Zamparette et al., 2020). MCR genes express a phosphoethanolamine transferase that catalyses lipid A to be modified, giving rise to resistance against polymyxins (Hussein et al., 2018; Vieira, 2024; Zamparette et al., 2020). The spreading of such mobile genetic elements across various species of bacteria and environments gives a great threat to the clinical utility of polymyxins, as indicated by Vieira in 2024, and by Zamparette et al. in 2020.

Table 1: Mechanisms of Polymyxin Resistance.

Mechanism	Description	References
Lipid A modification	Addition of L-Ara4N or pEtN reduces binding affinity of polymyxins.	Han et al., 2017; Deris et al., 2014
Efflux pumps	Actively expel polymyxins out of bacterial cells.	Gao et al., 2023
Plasmid-mediated mcr genes	Encode enzymes that modify lipid A, conferring resistance.	Hussein et al., 2018; Vieira, 2024
Biofilm formation	Increases bacterial resistance by reducing drug permeability.	Field et al., 2016

There are various mechanisms through which the hospital environment causes the emergence and spread of polymyxin-resistant strains. The high usage of polymyxins, including polymyxin B and colistin as a "last-resort" treatment for infections caused by multidrug-resistant Gram-

negative pathogens, has led to the selection of polymyxin-resistant strains (Qian et al., 2012). Studies have been carried out and proved there is a correlation with polymyxin usage and resistance of carbapenem-resistant Klebsiella pneumoniae in the hospital setting; however, it has been conflicting at times (Xiao et al., 2022; Xiao et al., 2023). Other mechanisms of resistance that would be seen include β-lactamases production and efflux pumps, with the subsequent emergence of polymyxin resistance mechanisms (Gao et al., 2023). These can result from horizontal gene transfer or chromosomal mutation. These processes are enhanced by the high density of microorganisms and frequent antibiotic exposure in the hospital setting (Abraham & Kwon, 2009).

The other reason for the emergence and spread of drug-resistant strains is said to be the lack of understanding and education of health personnel regarding antimicrobial resistance and stewardship (Abera et al., 2014). Poor infection control behaviors, including inadequate hand washing and disinfection of instruments, can further contribute to such cross-transmission in healthcare settings (Júnior et al., 2022). Apart from this, the hospital setting itself becomes a reservoir because resistant polymyxin-producing bacteria can easily reside on devices present within patient rooms, medical tools and equipment, and even the sewages of the hospital according to Ra (2023). Spread from the environment of the health care to the community contributes to the dissemination of resistant polymyxins. These factors may even worsen the situation by establishing an environment where polymyxin-resistant strains can be selected and transmitted when dealing with invasive medical devices and patients who are immunocompromised in a hospital where other multi-drug-resistant pathogens are abundant (Xiao et al., 2022; Xiao et al., 2023; Gao et al., 2023).

3. Current Challenges in Treating Polymyxin-Resistant Infections

First, the fast increase in enzymes, including extended-spectrum beta-lactamases and carbapenemases, has reversed the tide of success of most traditional therapies for severe Gram-negative infections. It therefore leaves the patients with fewer options, and there is an unprecedented level of resistance documented to last-line antibiotics, such as polymyxins (Oliveira et al., 2020). Mortality rates due to severe carbapenem-resistant and ESBL-producing Enterobacterales and Acinetobacter baumannii have also reached greater than 40% and 35%, respectively. There is a medical need not yet met by the development of new, safer polymyxin lipopeptide antibiotics to combat the increasing incidence of multidrug-resistant (MDR) pathogens, particularly in hospitals (Deris et al., 2014). The polymyxins have emerged as an essential part of the arsenal of antibiotics because they just so happen to be part of the very few, or sometimes only, antimicrobial drugs that are active against the multi-drug-resistant Gram-negative pathogens often giving rise to life-threatening infections among the most susceptible groups of patients (Field et al., 2016). However, clinical reports still abound in support of evidence that the Gram-negative bacteria also obtained resistance to the polymyxins (Field et al., 2016).

The development of polymyxin resistance is significant because the alternative effective antimicrobials are limited in supply (Li et al., 2022). It has been shown that the loss of

lipopolysaccharide from the outer membrane of Acinetobacter baumannii alters the antibiotic resistance profile, increasing sensitivity to a range of other antibiotics but increasing resistance to colistin and polymyxin B (Moffatt et al., 2010). Additionally, their ability to form biofilms tips it towards resistance because most biofilms are impermeable and notoriously hard to treat (Field et al., 2016). Such combinations have been shown to achieve effective reductions of the MIC of polymyxin B against several polymyxin-resistant strains (Zimmerman et al., 2020). However, some of these combinations could not suppress all the tested polymyxin-resistant isolates, and new studies on treatment options must be conducted (Zimmerman et al., 2020).

4. **Synergistic Approaches in Combatting**

Resistance Combination Therapies:

Synergistic drug interactions: Combination treatments can be made to leverage synergies among different antibiotic classes by augmenting the combined antihuman drug effect of drugs. As an illustration, the drug interaction involving daptomycin plus fosfomycin was synergistically bactericidal towards strains of gentamicin-resistant Enterococcus faecalis (Rice et al., 1989). As well, there has been observed synergy between β-lactam antibiotics and glycopeptide antibiotics against glycopeptide-resistant enterococci (Leclercq et al., 1991). These combinations are likely to better exploit combination treatments to override resistance pathways. This reduces the formation of resistant cells and can even prevent or delay the onset of resistant cell production if the individual drugs do not add together synergistically. Some in vitro experiments reported that, conversely, drug interactions showing antagonistic or suppressive effects might even be stronger in blocking multi-drug resistance development than synergistic combinations are (Ankomah et al., 2013). Also, the rapid change in drug combinations that favour various resistance phenotypes would lead to greater virus repression as well as delaying the manifestation of resistant phenotypes (Balzarini & Clercq, 2001).

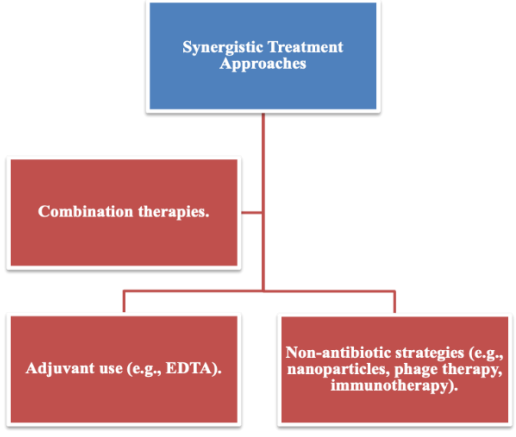

Figure 2. Summary of Synergistic Treatment Approaches.

Addressing heterogeneous populations: combination therapies target heterogeneous populations where resistant populations could be integrated due to drugs working on different mechanistic properties. It has a greater chance of killing all resistant cells, those with different resistance mechanisms, and decreases the chances of resistant strains (Mokhtari et al., 2017). Lowering drug concentrations: Combination therapies can sometimes permit a reduction in doses of individual drugs while still maintaining their efficacy, thereby reducing side effects associated with higher drug concentrations. This is very helpful in infections related to resistant strains where greater concentration levels of drugs are necessary (Mokhtari et al., 2017). Adjuvants: Adjuvants, such as EDTA, can be used to re-activate the action of polymyxin by disrupting the bacterial cell membrane. The synergistic antibacterial activity of polymyxin B and EDTA was shown against Pseudomonas aeruginosa and Staphylococcus aureus (Wu, 2023; Hale, 2024). EDTA is said to chelate calcium and magnesium ions from the lipopolysaccharide layer, which destabilizes the outer membrane and enhances the permeability of polymyxins (Komaniecka et al., 2016). A possible combination for enhancing the effectiveness of this antibiotic is through β-lactamase inhibitors that help inhibit the action of those degradative enzymes implicated in conferring resistance to polymyxins. A clear case on that effect of that combination is where it had proven to be synergistic between fosfomycin and polymyxin B against KPC-2-producing Klebsiella pneumoniae isolates (Ribeiro et al., 2023). The reconstitution of effectiveness of that use of the β-lactamase inhibitor, fosfomycin, against antibiotic-resistant Gram-negative pathogens is also possible.

Efflux pump inhibitors could revive the activities of polymyxins as the efflux mechanism that would expel them out of the bacterial cell would be inhibited. It has been reported that combining colistin with the efflux pump inhibitor otilonium bromide synergizes to significantly improve antimicrobial activity against persister Gram-negative bacterial pathogens (Chen et al., 2022). Some other adjuvants are known to rescue the anti-Staphylococcus activity against polymyxins (Wang et al., 2023). These most probably work through various mechanisms such as compromising cell membrane integrity or interference with resistance mechanisms. However, the species of bacteria and mechanisms of resistance may influence adjuvant selection and effectiveness. Adequate evaluation and adjuvant-polymyxin titration is essential for identifying effective treatment modalities against multidrug-resistant Gram-negative bacteria (Zimmerman et al., 2020; Yoshino et al., 2013; Esposito et al., 2017).

Non-Antibiotic Strategies:

There are still other approaches, like phage therapy, antimicrobial peptides, and nanoparticles, that might make it possible to use it in combination with polymyxin therapy for carbapenem-resistant organism infection treatment (Tang et al., 2022). Polymyxin therapy, for instance, has provided a cure for infections with several MDR Gram-negative bugs, such as Acinetobacter baumannii and Pseudomonas aeruginosa (Biagi et al., 2019). However, polymyxins were associated with significant toxicity, particularly nephrotoxicity and neurotoxicity (Biagi et al., 2019). Using polymyxin therapy in combination with other antimicrobial approaches may limit such adverse effects while improving clinical outcomes.

Phage therapy, with its promise as an adjunct to polymyxin therapy. In one experiment, addition of a specific phage, SA11, to polymyxin B displayed an additive antimicrobial activity against Staphylococcus aureus that comprises drug-resistant strains (Jo et al., 2016). The potential could be for increased effectiveness of polymyxins versus MDR Gram-negative bacteria. Another class of antimicrobials whose potential to complement polymyxin therapy includes another class of antimicrobials known as antimicrobial peptides (AMPs), which can interfere with a bacterial cell membrane by different means than polymyxins. A good example is where this research may be helpful in developing more novel AMPs by the exploration of the regulatory systems used by the bacteria to adapt to AMPs, for example, CprRS system in Pseudomonas aeruginosa (Fernández et al., 2012).

Another nanoparticle-based approach which has been studied is that of cubosomes loaded with polymyxin. Cubosomes are nanoparticles of lipid nature like the structure of polymyxins, capable of causing harm to Gram-negative bacteria's outer membrane. It has been observed that when these polymyxin-loaded cubosomes are combined, significantly greater antimicrobial activity was exhibited towards MDR bacteria compared with polymyxin alone and when it was in combination with cubosomes (Lai et al., 2022). This multi-therapy could potentially solve the problem of resistance and might improve the treatment with polymyxin.

Besides these antimicrobial strategies, the route of administration of polymyxins can also be optimized as an adjunct to its use. Inhaled or nebulized polymyxin therapy has been explored as a strategy for achieving high local concentrations in the lungs, which can be particularly useful in the treatment of VAP caused by MDR Gram-negative bacteria (Hasan et al., 2021; Wu, 2023). Other studies have demonstrated that supplementing aerosolized polymyxin B with intravenous polymyxin B helps improve clinical results for patients with low polymyxin exposure (Tang et al., 2022; Wu, 2023).

Immunotherapy:

Some works have been presented exploring how these approaches might strengthen the host's immune response and potentially overcome the problem posed by drug-resistant bacterial strains. An important key aspect is using mAbs that target specific epitopes or virulence factors from the pathogen. Xiong et al. 2017 had shown that a human biofilm-disrupting mAb was able to enhance the antibiotic efficacy in rodent models of infections due to Staphylococcus aureus and Acinetobacter baumannii. Such a mAb disrupted the biofilms produced by bacteria, the greatest factor responsible for resistance towards the antibiotics. Likewise, Pennini et al. Pennini et al. (2017) found that mAbs targeting the O-antigen of Klebsiella pneumoniae could provide protection and synergistic activity when used in combination with antibiotics, even against highly carbapenem-resistant strains.

Immune modulators, including microRNAs, might also be involved in regulating host immune responses to bacterial pathogens. Mishra et al. Mishra et al. (2021) reviewed the role of miR-30e-5p in the regulation of SOCS1 and SOCS3; these are key negative regulators of cytokine signaling. Their results indicate that inhibiting these microRNAs may be a useful method for enhancing innate host defense against bacterial infections. The bispecific antibodies, as proven by Patel et al. (Patel et al., 2017), could be used to target bacterial infections with alternative

approaches. Their research found that a bispecific DNA encoded IgG antibody was protective against Pseudomonas aeruginosa in a pneumonia challenge model, indicating the possibility of the technology.

Importantly, development of mAbs and immune modulators carries several benefits over traditional antibiotic therapies. According to Rossmann et al. (2015), mAbs can be administered prophylactically and provide immediate protection, while vaccines take some time before the host develops its protective antibodies. Moreover, the utilization of mAbs can help mitigate the detrimental effects of disruption in the beneficial microbiome as well as the spread of cross-resistance, which commonly accompany the use of antibiotics.

Table 2: Current Treatment Strategies for Polymyxin-Resistant Infections.

Strategy	Description	References
Combination therapy	Use of polymyxins with β-lactams, rifampin, or adjuvants like EDTA.	Ribeiro et al., 2023; Quale et al., 2012
Non-antibiotic approaches	Nanoparticles, phage therapy, and host-based therapies to enhance efficacy.	Lai et al., 2022; Tang et al., 2022
Rapid diagnostics	MALDI-TOF, PCR, and Rapid Polymyxin NP for early detection and management.	Nordmann et al., 2016; Fahim et al., 2023
Immunotherapy	Monoclonal antibodies targeting bacterial virulence factors.	Xiong et al., 2017

5. Role of Diagnostics and Genomics

Modern swift detection methods, including MALDI-TOF mass spectrometry and molecular assays, have improved significantly the identification of polymyxin-resistant isolates (Nordmann et al., 2016; Fahim et al., 2023; Nordmann et al., 2016). MALDI-TOF mass spectrometry is one of the techniques, quite powerful in identifying the species of bacteria, as well as to identify quite quickly and readily specific mechanisms of resistance, among which, notably, polymyxin resistance was mentioned by Nordmann et al. (2016); Nordmann et al., 2016). It is based on the examination of protein profiles of bacterial isolates, leading to rapid identification and differentiation of polymyxin-resistant strains (Nordmann et al., 2016;, Nordmann et al., 2016).

In addition to that, there are molecular tests, PCR-based tests included, developed to identify the presence of polymyxin resistance genes such as the mcr genes (Daly et al., 2017;, Sekyere, 2018). Results are within hours of several hours and so fast for identifying the isolates polymyxin-resistant. Compared to the regular susceptibility testing, like broth microdilution, which can take several days, the quick diagnostic tests, such as Rapid Polymyxin NP, results in 2 hours at minimum (Nordmann et al., 2016; Fahim et al. 2023). This test will determine the growth of bacteria in the presence of polymyxins; therefore, the strains could be identified rapidly as polymyxin-resistant (Nordmann et al., 2016; Fahim et al., 2023).

The Rapid Polymyxin NP test has been shown to be highly sensitive (99-99.3%) and specific (82-95.4%) in detecting polymyxin resistance, thus becoming a reliable alternative to the gold

standard broth microdilution method (Nordmann et al., 2016; Fahim et al., 2023;, Nordmann et al., 2016). This test would be very useful in resource-limited settings where access to more sophisticated susceptibility testing methods might be limited (Fahim et al., 2023). Apart from the rapid identification, incorporation of these diagnostic tools might also lead to prompt implementation of appropriate infection control practices, such as segregation of patients that harbor polymyxin-resistant isolates from other patients in order not to further disseminate such resistant organisms (Nordmann et al., 2016; Daly et al., 2017). In addition, the detection of rapid polymyxin resistance will help in choosing the appropriate antibiotic therapy and thus administer the patients with the most effective treatment as possible with reduced risk for treatment failure (Nordmann et al., 2016; Daly et al., 2017; Tsuji et al., 2016).

6. Infection Control Measures in Hospital Settings

Combination therapy that targets multiple resistance mechanisms: Combination therapy that targets heterogeneous resistance mechanisms in Gram-negative bacteria has been found to be more effective than monotherapy as reported by Zimmerman et al. 2020; Combining polymyxins with other antibiotics such as amikacin is known to reduce mortality compared to polymyxin monotherapy as reported by Xia & Jiang 2021.

Table 3: Clinical Outcomes of Combination Therapies.

Combination	Outcome	References
Polymyxin B + fosfomycin	Synergistic effects against KPC-2-producing Klebsiella pneumoniae.	Ribeiro et al., 2023
Polymyxin B + EDTA	Enhanced permeability and antibacterial activity.	Hale, 2024
Polymyxin B + resveratrol	Reduced MICs and improved anti-biofilm activity.	Liu et al., 2020
Polymyxin B + rifampin	Potentially improved outcomes in animal models.	Quale et al., 2012
Polymyxin B + nanoparticles	Significant antimicrobial activity against MDR strains.	Lai et al., 2022

Decolonization of skin by chlorhexidine: Chlorhexidine bathing would also reduce catheter colonization and the incidence of catheter-associated bloodstream infections because of polymyxin-resistant strains. The MIC of polymyxin-resistant isolates of Acinetobacter baumannii was reported to be less than that contained in the bathing solution of chlorhexidine (Carrasco et al., 2021).

Monitoring of polymyxin resistance: Since the emergence of polymyxin resistance is time-dependent, the incidence of polymyxin resistance needs to be monitored and tracked in hospitals. An increasing trend occurs with an increase in time since infection with polymyxin-resistant Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii (Xiao et al., 2023; Xiao et al., 2022; Roy et al., 2018). Trends may be useful in making the management decisions.

Antimicrobial stewardship: Use of polymyxins and other last-resort antibiotics will be done properly if the programs of antimicrobial stewardship are implemented. The programs would

prevent further development and spread of resistance (Qian et al., 2012). Monotherapy with polymyxins must be limited by giving alternative antimicrobial options as well as combination therapy.

Infection Prevention and Control: Standard precautions: hygiene, contact precautions, cleaning the environment, proper devices, all of these with a goal of preventing nosocomial spread of polymyxin-resistant strains in the health institution (Deris et al., 2014; Tran et al., 2015).

7. Case Studies and Clinical Outcomes

One such example is the co-administration of polymyxin B with the antineoplastic drug mitotane, which has been proved to cause synergistic bacterial killing and inhibit regrowth in the multidrug-resistant Acinetobacter baumannii strains (Tran et al., 2018). In this study, it was demonstrated that both polymyxin-susceptible and -resistant isolates of A. baumannii were affected. The other important combination is the polymyxin B with anthelmintic drug closantel. It has proved that it was actually a viable combination as it showed an improved bactericidal activity of polymyxin B on multidrug-resistant A. baumannii (Tran et al., 2015). It was also proved in this experiment that closantel fully inhibited the regrowth seen when administered alone with polymyxin B.

Another was the polyphenolic compound resveratrol, which was seen to augment the antimicrobial activity of polymyxin B against Klebsiella pneumoniae and Escherichia coli isolates resistant to polymyxin B. The combination resulted in an appreciable decrease in MIC of polymyxin B (Liu et al., 2020). Another synergistic agent identified was the combination of polymyxin B and the antibiotic fusidic acid, which showed potent effects against isolates of K. pneumoniae and E. coli, especially those resistant to polymyxin B (Chen, 2023). It was more effective than the use of either agent alone in treating such infections.

In addition, the ionophore PBT2 was demonstrated to increase the disruption of polymyxin resistance in polymyxin-resistant K. pneumoniae, E. coli, A. baumannii, and Pseudomonas aeruginosa (Oliveira et al., 2020). This repurposed drug provides a potential alternative to de novo drug discovery to combat antimicrobial resistance. Although animal models seem to demonstrate improved outcomes with polymyxin B combined with rifampin, controlled studies in humans are still limited (Quale et al., 2012). The combination of polymyxins with other antibiotics is seriously plagued with regrowth, which is often a very troublesome issue with monotherapy with any of the polymyxins.

8. Prospects in Fighting Polymyxin Resistance

AMS Programs: The development of adequate AMS programs would complement new antimicrobials. AMS programs would reserve the potency of currently prescribed therapies and, therefore, the highest clinical outcomes for the patients. AMS strategies range from prudent use of antimicrobials, developing expedited diagnostics to enable more targeted utilization of

antibiotics based on causative agents, and restriction in usage of broad-spectrum antibiotics (Matsumoto et al., 2022).

Exploration of New Mechanisms of Antimicrobic Activity: There is also a need for more study and discovery of new antimicrobic medications and other treatments, based on multitarget mechanisms (Vaishampayan & Grohmann, 2021; Lázár et al., 2018). These might be ROS-generating antimicrobics, antimicrobial peptides, and peptidomimetic drugs which are less likely to confer resistance (Vaishampayan & Grohmann, 2021; Lázár et al., 2018).

Biofilm-Related Infections: New antimicrobial agents and treatment strategies are required to be developed against infections by antibiotic-resistant and biofilm-forming bacteria (Vizzarro & Jacquier, 2022). Biofilms are challenging because they show higher antibiotic tolerance than planktonic bacteria (Vizzarro & Jacquier, 2022).

Host-based Therapies: With the development of host-based therapies, which rely on activating microbicidal responses in macrophages and neutrophils, a new paradigm is growing as an alternative to fight antimicrobial resistance (Watson et al., 2020). The strategy in such approaches would be to increase the host's immune response against infections.

Government Policy Interventions: The information-oriented strategies used in the interventions are public awareness campaigns and antimicrobial guidelines (Katwyk et al., 2019). The objectives of such government policy interventions are to raise awareness of health care professionals and the community about the resistance to antibiotics and its proper use of antibiotics (Katwyk et al., 2019).

Natural Resource Discovery: Filamentous ascomycetes such as Penicillium chrysogenum can be a very good source of antimicrobial biomolecules that may find more use in medical and agricultural applications (Huber et al., 2018). Similarly, anurans, or frogs, have also been very remarkable sources of biologically active compounds, which are mainly in the form of antimicrobial peptides and might find use in drug development (Freitas et al., 2023).

9. Global and Policy Implications

Various international policies and guidelines cover the management of polymyxin-resistant infections. World Health Organization's Global Action Plan on Antimicrobial Resistance aids countries in addressing this problem, which is faced around the world, by devising strategies to maintain the potency of polymyxins: "Global Action Plan on Antimicrobial Resistance", 2015. The use of polymyxin-based combinations is very common because of the increasing resistance to polymyxins, thereby improving the antimicrobial activity and reducing resistance development. However, evidence-based treatment guidelines for these combinations are lacking, indicating the need for optimization (Wickremasinghe et al., 2021).

Many tests have been developed and assessed for detecting polymyxin resistance. Some of the tested methods include Etest, elution, agar dilution, polymyxin NP, and automated systems; all have very important limitations. BMD is the gold standard method for establishing polymyxin susceptibility; however, it is cumbersome and time-consuming (Perez et al., 2020). Polymyxins, such as polymyxin B or colistin, are also known to cause the enrichment of polymyxin-resistant

subpopulations during monotherapy. Thus, there is a warning for the use of these drugs in treating Acinetobacter baumannii infections. Combination therapies, such as the combination of polymyxin B and the anthelmintic closantel, resulted in improved bacterial killing of multidrug-resistant A. baumannii (Tran et al., 2015).

The mechanisms of resistance to polymyxin B in Pseudomonas aeruginosa are not well understood, though the most common mechanism has been reported to be the alteration of the lipid A component of the outer membrane lipopolysaccharide, the initial target of polymyxin B. Combination therapies, like polymyxin B and resveratrol, were recently shown to have synergistic antibacterial and anti-biofilm activities against multidrug-resistant P. aeruginosa (Qi et al., 2022). The Swedish policies in relation to the containment of antibiotic resistance are aligned with the resolution of the World Health Assembly and the Global Action Plan on Antimicrobial Resistance. The challenge of antibiotic resistance is a complex global issue that requires international collaboration. Coordination and synergies among innovators, researchers, funders, and policymakers are necessary to handle the issue of antibiotic resistance (Eriksen et al., 2021).

This hike in antibiotic consumption around the world and geographical convergence of consumption between the year 2000 and 2015 has contributed to the surge in antibiotic resistance. The global response to nearly untreatable infections that emerged and spread remains slow and inadequate (Klein et al., 2018). Other international efforts include the development of several initiatives aimed at combating antibiotic resistance. Among them are TATFAR, GARP, GHSA, the high-level United Nations meeting regarding AMR, the One Health Approach, and AMR Challenge 2018-2019. Each effort was established to create ideas of policy propositions, enhance health standards in the community, and unite global interventions toward halting the increase of antibiotics resistant strains (Domínguez et al., 2021).

Polymyxins have become one of the most important last-line therapies against multidrug-resistant Gram-negative pathogens due to their resistance to most or all other antibiotic classes. However, the use of polymyxins is limited by nephrotoxicity and emergence of resistance, thus forming the basis for the development of polymyxin analogues with reduced toxicity and approaches to minimize emergence of polymyxin resistance (Quale et al., 2012). Though polymyxins like polymyxin B and colistin have resurfaced as treatments for infections of multidrug-resistant Gram-negative bacteria, including carbapenem-resistant pathogens, a significant concern is the rising rate of polymyxin resistance. Combination therapies, among others, which include the administration of polymyxin and vancomycin, have displayed synergistic effects against carbapenem- and polymyxin-resistant Klebsiella pneumoniae (Nwabor, 2023).

10. Conclusion

The main challenge in fighting infections by Mult resistant Gram-negative bacteria is resistance to polymyxins. Advanced resistance mechanisms require multifaceted solutions: novel antimicrobials, combination therapies, and advanced diagnostics. A new wave of adjuvants and

non-antibiotic strategies, including nanoparticles and phage therapy, promises to increase polymyxin efficacy while minimizing resistance. Proper and comprehensive antimicrobial stewardship programs along with strong infection control practices will help to halt the horizontal spread of resistant strains in healthcare settings. Also, more rapid diagnostic tools like MALDI-TOF and molecular assays would help its detection in time with effective treatment. Host-based therapies like monoclonal antibodies also offer an armament against such infections. The urgent call is to continue research and cooperative worldwide support, with proper government policy backing, for combat against this growing polymyxin resistance crisis so as not to lose such extremely useful last alternatives to treat these high-risk patients with proper antimicrobial coverages.

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Author contributions

The first author wrote the original text, however all authors contributed significantly through data collection and literature searches. Each author accepted responsibility for all aspects of the work, took part in the critical revision of the book, and gave their approval to the final draft.

Conflict of Interest

Authors declare they don’t have any conflict of interest.

Ethical Approval

Not Applicable

WORKS CITED

Moffatt, J., Harper, M., Harrison, P., Hale, J., Vinogradov, E., Seemann, T., … & Boyce, J. (2010). Colistin resistance in acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. Antimicrobial Agents and Chemotherapy, 54(12), 4971-4977. https://doi.org/10.1128/aac.00834-10

Oliveira, D., Bohlmann, L., Conroy, T., Jen, F., Everest-Dass, A., Hansford, K., … & Walker, M. (2020). Repurposing a neurodegenerative disease drug to treat gram-negative antibiotic-resistant bacterial sepsis. Science Translational Medicine, 12(570). https://doi.org/10.1126/scitranslmed.abb3791

Perez, L., Carniel, E., Narváez, G., & Dias, C. (2020). Evaluation of a polymyxin drop test for polymyxin resistance detection among non-fermentative gram-negative rods and enterobacterales resistant to carbapenems. Apmis, 129(3), 138-142. https://doi.org/10.1111/apm.13096

Qi, L., Li, F., Duan, J., Song, S., Pan, Y., Liu, H., … & Li, L. (2022). Synergistic antibacterial and anti-biofilm activities of resveratrol and polymyxin b against multidrug-resistant

pseudomonas aeruginosa. The Journal of Antibiotics, 75(10), 567-575. https://doi.org/10.1038/s41429-022-00555-1

Tran, T., Cheah, S., Yu, H., Bergen, P., Nation, R., Creek, D., … & Li, J. (2015). Anthelmintic closantel enhances bacterial killing of polymyxin b against multidrug-resistant acinetobacter baumannii. The Journal of Antibiotics, 69(6), 415-421. https://doi.org/10.1038/ja.2015.127

Xu, X., Zhu, R., Lian, S., Zhang, H., Chen, X., Fan, L., … & Cao, Y. (2022). Risk factors and molecular mechanism of polymyxin b resistance in carbapenem-resistant klebsiella pneumoniae isolates from a tertiary hospital in fujian, china. Infection and Drug Resistance, Volume 15, 7485-7494. https://doi.org/10.2147/idr.s391674

Yu, Z., Qin, W., Lin, J., Fang, S., & Qiu, J. (2015). Antibacterial mechanisms of polymyxin and bacterial resistance. Biomed Research International, 2015, 1-11. https://doi.org/10.1155/2015/679109

Arroyo, L., Herrera, C., Fernández, L., Hankins, J., Trent, M., & Hancock, R. (2011). The pmrcaboperon mediates polymyxin resistance in acinetobacter baumanniiatcc 17978 and clinical isolates through phosphoethanolamine modification of lipid a. Antimicrobial Agents and Chemotherapy, 55(8), 3743-3751. https://doi.org/10.1128/aac.00256-11

Gao, W., Li, C., Wang, F., Wang, Z., Chen, X., Tan, M., … & Zong, G. (2023). Efflux pump in genomic island gi-m202a mediates transfer of polymyxin b resistance in pandoraea pnomenusa m202.. https://doi.org/10.21203/rs.3.rs-2606888/v1

Maifiah, M., Cheah, S., Johnson, M., Han, M., Boyce, J., Thamlikitkul, V., … & Li, J. (2016). Global metabolic analyses identify key differences in metabolite levels between polymyxin-susceptible and polymyxin-resistant acinetobacter baumannii. Scientific Reports, 6(1). https://doi.org/10.1038/srep22287

Qian, C., Wu, X., Teng, Y., Zhao, W., Ou, L., Fang, S., … & Gao, H. (2012). Battacin (octapeptin b5), a new cyclic lipopeptide antibiotic from paenibacillus tianmuensis active against multidrug-resistant gram-negative bacteria. Antimicrobial Agents and Chemotherapy, 56(3), 1458-1465. https://doi.org/10.1128/aac.05580-11

Tsuji, B., Landersdorfer, C., Lenhard, J., Cheah, S., Thamlikitkul, V., Rao, G., … & Li, J. (2016). Paradoxical effect of polymyxin b: high drug exposure amplifies resistance in acinetobacter baumannii. Antimicrobial Agents and Chemotherapy, 60(7), 3913-3920. https://doi.org/10.1128/aac.02831-15

Velkov, T., Roberts, K., Nation, R., Wang, J., Thompson, P., & Li, J. (2014). Teaching ‘old’ polymyxins new tricks: new-generation lipopeptides targeting gram-negative ‘superbugs’. Acs Chemical Biology, 9(5), 1172-1177. https://doi.org/10.1021/cb500080r

Xiao, C., Li, X., Huang, L., Cao, H., Han, L., Ni, Y., … & Yang, Z. (2023). Prevalence and molecular characteristics of polymyxin-resistant enterobacterales in a chinese tertiary teaching hospital. Frontiers in Cellular and Infection Microbiology, 13. https://doi.org/10.3389/fcimb.2023.1118122

Zimmerman, S., Lafontaine, A., Herrera, C., McLean, A., & Trent, M. (2020). A whole-cell screen identifies small bioactives that synergize with polymyxin and exhibit antimicrobial activities against multidrug-resistant bacteria. Antimicrobial Agents and Chemotherapy, 64(3). https://doi.org/10.1128/aac.01677-19

Deris, Z., Swarbrick, J., Roberts, K., Azad, M., Akter, J., Horne, A., … & Li, J. (2014). Probing the penetration of antimicrobial polymyxin lipopeptides into gram-negative bacteria. Bioconjugate Chemistry, 25(4), 750-760. https://doi.org/10.1021/bc500094d

Han, M., Velkov, T., Zhu, Y., Roberts, K., Brun, A., Chow, S., … & Li, J. (2017). Polymyxin-induced lipid a deacylation in pseudomonas aeruginosa perturbs polymyxin penetration and

confers high-level resistance. Acs Chemical Biology, 13(1), 121-130. https://doi.org/10.1021/acschembio.7b00836

Hussein, M., Han, M., Zhu, Y., Schneider-Futschik, E., Hu, X., Zhou, Q., … & Velkov, T. (2018). Mechanistic insights from global metabolomics studies into synergistic bactericidal effect of a polymyxin b combination with tamoxifen against cystic fibrosis mdr pseudomonas aeruginosa. Computational and Structural Biotechnology Journal, 16, 587-599. https://doi.org/10.1016/j.csbj.2018.11.001

Vieira, T. (2024). Polymyxin resistance in salmonella: exploring mutations and genetic determinants of non-human isolates. Antibiotics, 13(2), 110. https://doi.org/10.3390/antibiotics13020110

Zamparette, C., Schörner, M., Campos, E., Moura, Q., Cerdeira, L., Tartari, D., … & Sincero, T. (2020). Incx4 plasmid-mediated mcr-1.1 in polymyxin-resistant escherichia coli from outpatients in santa catarina, southern brazil. Microbial Drug Resistance, 26(11), 1326-1333. https://doi.org/10.1089/mdr.2019.0203

Abera, B., Kibret, M., & Mulu, W. (2014). Knowledge and beliefs on antimicrobial resistance among physicians and nurses in hospitals in amhara region, ethiopia. BMC Pharmacology and Toxicology, 15(1). https://doi.org/10.1186/2050-6511-15-26

Abraham, N. and Kwon, D. (2009). A single amino acid substitution in pmrb is associated with polymyxin b resistance in clinical isolate ofpseudomonas aeruginosa. Fems Microbiology Letters, 298(2), 249-254. https://doi.org/10.1111/j.1574-6968.2009.01720.x

Júnior, S., Campos, L., Cavalcanti, I., Costa, J., Ferreira, G., Silva, J., … & Oliveira, M. (2022). Dissemination and prevalence of polymyxin-resistant bacteria of clinical and environmental origin. Research Society and Development, 11(13), e94111334996. https://doi.org/10.33448/rsd-v11i13.34996

Ra, A. (2023). Ceftazidime-avibactam alone or in combination with aztreonam versus polymyxins in the management of carbapenem-resistant klebsiella pneumoniae nosocomial infections (capri study): a retrospective cohort study from south india.. https://doi.org/10.21203/rs.3.rs-2842454/v1

Xiao, C., Zhu, Y., Yang, Z., Shi, D., Ni, Y., Li, H., … & Li, J. (2022). Prevalence and molecular characteristics of polymyxin-resistant pseudomonas aeruginosa in a chinese tertiary teaching hospital. Antibiotics, 11(6), 799. https://doi.org/10.3390/antibiotics11060799

Field, D., Seisling, N., Cotter, P., Ross, R., & Hill, C. (2016). Synergistic nisin-polymyxin combinations for the control of pseudomonas biofilm formation. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.01713

Li, J., Tang, M., Xia, F., Min, C., Hu, Y., Wang, H., … & Zou, M. (2022). Emergence of polymyxin b-heteroresistant hypervirulent klebsiella pneumoniae from an individual in the community with asymptomatic bacteriuria. BMC Microbiology, 22(1). https://doi.org/10.1186/s12866-022-02462-9

Ankomah, P., Johnson, P., & Levin, B. (2013). The pharmaco –, population and evolutionary dynamics of multi-drug therapy: experiments with s. aureus and e. coli and computer simulations. Plos Pathogens, 9(4), e1003300. https://doi.org/10.1371/journal.ppat.1003300

Balzarini, J. and Clercq, E. (2001). Rapid alternation of drug therapy is highly efficient in suppressing the emergence of mutant drug-resistant hiv strains in cell culture. Aids Research and Human Retroviruses, 17(17), 1625-1634. https://doi.org/10.1089/088922201753342031

Leclercq, R., Bingen, E., Su, Q., Lambert-Zechovski, N., Courvalin, P., & Duval, J. (1991). Effects of combinations of beta-lactams, daptomycin, gentamicin, and glycopeptides

against glycopeptide-resistant enterococci. Antimicrobial Agents and Chemotherapy, 35(1), 92-98. https://doi.org/10.1128/aac.35.1.92

Mokhtari, R., Homayouni, T., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., … & Yeger, H. (2017). Combination therapy in combating cancer. Oncotarget, 8(23), 38022-38043. https://doi.org/10.18632/oncotarget.16723

Rice, L., Eliopoulos, G., & Moellering, R. (1989). In vitro synergism between daptomycin and fosfomycin against enterococcus faecalis isolates with high-level gentamicin resistance. Antimicrobial Agents and Chemotherapy, 33(4), 470-473. https://doi.org/10.1128/aac.33.4.470

Chen, X., Liu, C., Chen, K., Zeng, P., Chan, E., & Chen, S. (2022). Otilonium bromide boosts antimicrobial activities of colistin against gram-negative pathogens and their persisters. Communications Biology, 5(1). https://doi.org/10.1038/s42003-022-03561-z

Esposito, F., Fernandes, M., Lopes, R., Muñoz, M., Sabino, C., Cunha, M., … & Lincopán, N. (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. https://doi.org/10.1128/jcm.00835-17

Hale, S. (2024). Polymyxin b and ethylenediaminetetraacetic acid act synergistically against pseudomonas aeruginosa and staphylococcus aureus. Microbiology Spectrum. https://doi.org/10.1128/spectrum.01709-23

Komaniecka, I., Zamlynska, K., Zan, R., Staszczak, M., Pawelec, J., Seta, I., … & Choma, A. (2016). Rhizobium strains differ considerably in outer membrane permeability and polymyxin b resistance. Acta Biochimica Polonica, 63(3). https://doi.org/10.18388/abp.2015_1212

Ribeiro, Á., Chikhani, Y., Valiatti, T., Valêncio, A., Kurihara, M., Santos, F., … & Gales, A. (2023). In vitro and in vivo synergism of fosfomycin in combination with meropenem or polymyxin b against kpc-2-producing klebsiella pneumoniae clinical isolates. Antibiotics, 12(2), 237. https://doi.org/10.3390/antibiotics12020237

Wang, L., Sun, F., Gao, Y., Ji, Q., Fu, L., Liu, Z., … & Li, L. (2023). Synergistic effect of polymyxin b with baicalein against mcr-1 postive escherichia coli.. https://doi.org/10.21203/rs.3.rs-3004198/v1

Wu, Y. (2023). Simeprevir restores the anti-staphylococcus activity of polymyxins. Amb Express, 13(1). https://doi.org/10.1186/s13568-023-01634-8

Yoshino, N., Endo, M., Kanno, H., Matsukawa, N., Tsutsumi, R., Takeshita, R., … & Sato, S. (2013). Polymyxins as novel and safe mucosal adjuvants to induce humoral immune responses in mice. Plos One, 8(4), e61643. https://doi.org/10.1371/journal.pone.0061643 Biagi, M., Butler, D., Tan, X., Qasmieh, S., & Wenzler, E. (2019). A breath of fresh air in the fog of antimicrobial resistance: inhaled polymyxins for gram-negative pneumonia. Antibiotics, 8(1), 27. https://doi.org/10.3390/antibiotics8010027

Fernández, L., Jenssen, H., Bains, M., Wiegand, I., Gooderham, W., & Hancock, R. (2012). The two-component system cprrs senses cationic peptides and triggers adaptive resistance in pseudomonas aeruginosa independently of parrs. Antimicrobial Agents and Chemotherapy, 56(12), 6212-6222. https://doi.org/10.1128/aac.01530-12

Hasan, J., Rabbani, R., Anam, A., Santini, A., & Huq, S. (2021). The susceptibility of mdr-k. pneumoniae to polymyxin b plus its nebulised form versus polymyxin b alone in critically ill south asian patients. The Journal of Critical Care Medicine, 7(1), 28-36. https://doi.org/10.2478/jccm-2020-0044

Jo, A., Ding, T., & Ahn, J. (2016). Synergistic antimicrobial activity of bacteriophages and antibiotics against staphylococcus aureus. Food Science and Biotechnology, 25(3), 935-940. https://doi.org/10.1007/s10068-016-0153-0

Lai, X., Han, M., Ding, Y., Chow, S., Wu, C., Bergen, P., … & Shen, H. (2022). A polytherapy based approach to combat antimicrobial resistance using cubosomes. Nature Communications, 13(1). https://doi.org/10.1038/s41467-022-28012-5

Tang, T., Liu, Y., Xu, P., Zhong, Y., Yang, M., Ma, W., … & Zhang, B. (2022). Optimization of polymyxin b regimens for the treatment of carbapenem-resistant organism nosocomial pneumonia: a real-world prospective study.. https://doi.org/10.21203/rs.3.rs-2310566/v1

Wu, Z. (2023). Comparison of the clinical efficacy and toxicity of nebulized polymyxin monotherapy and combined intravenous and nebulized polymyxin for the treatment of ventilator-associated pneumonia caused by carbapenem-resistant gram-negative bacteria: a retrospective cohort study. Frontiers in Pharmacology, 14. https://doi.org/10.3389/fphar.2023.1209063

Mishra, R., Krishnamoorthy, P., & Kumar, H. (2021). Microrna-30e-5p regulates socs1 and socs3 during bacterial infection. Frontiers in Cellular and Infection Microbiology, 10. https://doi.org/10.3389/fcimb.2020.604016

Patel, A., DiGiandomenico, A., Keller, A., Smith, T., Park, D., Ramos, S., … & Weiner, D. (2017). An engineered bispecific dna-encoded igg antibody protects against pseudomonas aeruginosa in a pneumonia challenge model. Nature Communications, 8(1). https://doi.org/10.1038/s41467-017-00576-7

Pennini, M., Marco, A., Pelletier, M., Bonnell, J., Cvitkovic, R., Beltramello, M., … & Stover, C. (2017). Immune stealth-driven o2 serotype prevalence and potential for therapeutic antibodies against multidrug resistant klebsiella pneumoniae. Nature Communications, 8(1). https://doi.org/10.1038/s41467-017-02223-7

Rossmann, F., Laverde, D., Kropec, A., Romero-Saavedra, F., Meyer-Buehn, M., & Hüebner, J. (2015). Isolation of highly active monoclonal antibodies against multiresistant gram-positive bacteria. Plos One, 10(2), e0118405. https://doi.org/10.1371/journal.pone.0118405

Xiong, Y., Estellés, A., Li, L., Abdelhady, W., Gonzales, R., Bayer, A., … & Kauvar, L. (2017). A human biofilm-disrupting monoclonal antibody potentiates antibiotic efficacy in rodent models of both staphylococcus aureus and acinetobacter baumannii infections. Antimicrobial Agents and Chemotherapy, 61(10). https://doi.org/10.1128/aac.00904-17

Daly, S., Sturge, C., Felder-Scott, C., Geller, B., & Greenberg, D. (2017). Mcr-1 inhibition with peptide-conjugated phosphorodiamidate morpholino oligomers restores sensitivity to polymyxin in escherichia coli. Mbio, 8(6). https://doi.org/10.1128/mbio.01315-17

Fahim, N., El-Beih, A., & ElMasry, S. (2023). Evaluation of polymyxin np test as a rapid method for detection of polymyxins resistance in enterobacteriaceae . Afro-Egyptian Journal of Infectious and Endemic Diseases, 0(0), 0-0. https://doi.org/10.21608/aeji.2023.182460.1275

Nordmann, P., Jayol, A., & Poirel, L. (2016). 130 rapid detection of polymyxin resistance in enterobacteriaceae. Journal of Cystic Fibrosis, 15, S84. https://doi.org/10.1016/s1569-1993(16)30368-x

Nordmann, P., Jayol, A., & Poirel, L. (2016). Rapid detection of polymyxin resistance inenterobacteriaceae. Emerging Infectious Diseases, 22(6), 1038-1043. https://doi.org/10.3201/eid2206.151840

Sekyere, J. (2018). mcr colistin resistance gene: a systematic review of current diagnostics and detection methods. Microbiologyopen, 8(4). https://doi.org/10.1002/mbo3.682

Carrasco, L., Dabul, A., Boralli, C., Righetto, G., Carvalho, I., Dornelas, J., … & Camargo, I. (2021). Polymyxin resistance among xdr st1 carbapenem-resistant acinetobacter baumannii clone expanding in a teaching hospital. Frontiers in Microbiology, 12. https://doi.org/10.3389/fmicb.2021.622704

Roy, S., Paul, D., Elizabeth, R., Dhar, D., Chakarvarty, A., & Bhattacharjee, A. (2018). Transcriptional expression of pmrb and arna within polymyxin-resistant nosocomial isolates of pseudomonas aeruginosa from india. Journal of Microbiology and Infectious Diseases, 08(02), 61-68. https://doi.org/10.5799/jmid.434595

Xia, G. and Jiang, R. (2021). Efficacy and safety of polymyxin b in carbapenem-resistant gram-negative organisms infections. BMC Infectious Diseases, 21(1). https://doi.org/10.1186/s12879-021-06719-y

Chen, S. (2023). Polymyxin b and fusidic acid, a novel potent synergistic combination against klebsiella pneumoniae and escherichia coli isolates with polymyxin b resistance. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1220683

Liu, L., Yu, J., Shen, X., Cao, X., Zhan, Q., Guo, Y., … & Yu, F. (2020). Resveratrol enhances the antimicrobial effect of polymyxin b on klebsiella pneumoniae and escherichia coli isolates with polymyxin b resistance. BMC Microbiology, 20(1). https://doi.org/10.1186/s12866-020-01995-1

Quale, J., Shah, N., Kelly, P., Babu, E., Bäcker, M., Rosas-Garcia, G., … & Landman, D. (2012). Activity of polymyxin b and the novel polymyxin analogue cb-182,804 against contemporary gram-negative pathogens in new york city. Microbial Drug Resistance, 18(2), 132-136. https://doi.org/10.1089/mdr.2011.0163

Tran, T., Bergen, P., Creek, D., Velkov, T., & Li, J. (2018). Synergistic killing of polymyxin b in combination with the antineoplastic drug mitotane against polymyxin-susceptible and -resistant acinetobacter baumannii: a metabolomic study. Frontiers in Pharmacology, 9. https://doi.org/10.3389/fphar.2018.00359

Freitas, G., Barbosa, J., Santana, C., Magalhães, A., Macedo, K., Souza, J., … & Castro, M. (2023). Purification and biological properties of raniseptins-3 and -6, two antimicrobial peptides from boana raniceps (cope, 1862) skin secretion. Biomolecules, 13(3), 576. https://doi.org/10.3390/biom13030576

Huber, A., Hajdu, D., Bratschun-Khan, D., Gáspári, Z., Varbanov, M., Philippot, S., … & Batta, G. (2018). New antimicrobial potential and structural properties of pafb: a cationic, cysteine-rich protein from penicillium chrysogenum q176. Scientific Reports, 8(1). https://doi.org/10.1038/s41598-018-20002-2

Katwyk, S., Grimshaw, J., Nkangu, M., Nagi, R., Mendelson, M., Taljaard, M., … & Hoffman, S. (2019). Government policy interventions to reduce human antimicrobial use: a systematic review and evidence map. Plos Medicine, 16(6), e1002819. https://doi.org/10.1371/journal.pmed.1002819

Lázár, V., Martins, A., Spohn, R., Daruka, L., Grézal, G., Fekete, G., … & Pál, C. (2018). Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides. Nature Microbiology, 3(6), 718-731. https://doi.org/10.1038/s41564-018-0164-0

Matsumoto, T., Yuasa, A., Miller, R., Pritchard, C., Ohashi, T., Taie, A., … & Gordon, J. (2022). Estimating the economic and clinical value of introducing ceftazidime/avibactam into antimicrobial practice in japan: a dynamic modelling study. Pharmacoeconomics - Open, 7(1), 65-76. https://doi.org/10.1007/s41669-022-00368-w

Poon, S. (2023). Antimicrobial resistance research in singapore – mapping current trends and future perspectives.. https://doi.org/10.1101/2023.11.28.23299149

Vaishampayan, A. and Grohmann, E. (2021). Antimicrobials functioning through ros-mediated mechanisms: current insights. Microorganisms, 10(1), 61. https://doi.org/10.3390/microorganisms10010061

Vizzarro, G. and Jacquier, N. (2022). in vitro synergistic action of tat-rasgap317-326 peptide with antibiotics against gram-negative pathogens.. https://doi.org/10.1101/2022.09.26.509445

Watson, K., Russell, C., Baillie, J., Dhaliwal, K., Fitzgerald, J., Mitchell, T., … & Dockrell, D. (2020). Developing novel host-based therapies targeting microbicidal responses in macrophages and neutrophils to combat bacterial antimicrobial resistance. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.00786

(2015). Global action plan on antimicrobial resistance. Microbe Magazine, 10(9), 354-355. https://doi.org/10.1128/microbe.10.354.1

Domínguez, D., Chacón, L., & Wallace, D. (2021). Anthropogenic activities and the problem of antibiotic resistance in latin america: a water issue. Water, 13(19), 2693. https://doi.org/10.3390/w13192693

Eriksen, J., Björkman, I., Röing, M., Essack, S., & Lundborg, C. (2021). Exploring the one health perspective in sweden's policies for containing antibiotic resistance. Antibiotics, 10(5), 526. https://doi.org/10.3390/antibiotics10050526

Klein, E., Boeckel, T., Martínez, E., Pant, S., Gandra, S., Levin, S., … & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proceedings of the National Academy of Sciences, 115(15). https://doi.org/10.1073/pnas.1717295115

Nwabor, O. (2023). Synergistic effects of polymyxin and vancomycin combinations on carbapenem- and polymyxin-resistant klebsiella pneumoniae and their molecular characteristics. Microbiology Spectrum, 11(6). https://doi.org/10.1128/spectrum.01199-23

Wickremasinghe, H., Yu, H., Azad, M., Zhao, J., Bergen, P., Velkov, T., … & Li, J. (2021). Clinically relevant concentrations of polymyxin b and meropenem synergistically kill multidrug-resistant pseudomonas aeruginosa and minimize biofilm formation. Antibiotics, 10(4), 405. https://doi.org/10.3390/antibiotics10040405