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Original Research Article

In vitro and *in vivo* synergistic activity of antibiotic combinations against colistin-resistant *pseudomonas aeruginosa*: a study from a tertiary care hospital in Bangladesh

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ABSTRACT

Background: The emergence of colistin-resistant *Pseudomonas aeruginosa* poses a serious therapeutic challenge, particularly in low- and middle-income countries where treatment options are limited. Combination therapy is increasingly explored to improve clinical outcomes, but *in vivo* validation still remains insufficient.
Methods: A cross-sectional experimental study was conducted at a tertiary-care hospital in Bangladesh (2019). Clinical isolates of *P. aeruginosa* were identified and tested for antimicrobial susceptibility following CLSI guidelines. Colistin resistance was detected by agar dilution MIC testing and further confirmed by PCR. Molecular detection of colistin resistance–associated genes (*PMR A, PMR B, PMR C, PHO P, PHO Q* and *mcr-1-5*) was done by PCR. *In vitro* synergy of colistin–imipenem and colistin–amikacin combinations were evaluated by MIC reduction and fractional inhibitory concentration index (FICI). A murine infection model was used to assess the therapeutic efficacy.
Results: Among the 63 isolates of *P. aeruginosa,* 12 (19.5%) were identified as colistin-resistant. Multidrug resistance was observed among all the isolates, with high-level colistin MICs (≥4 µg/ml). Chromosomally mediated resistance genes (PMR and PHO systems) were only detected instead of any plasmid-mediated mcr genes. *In vitro* synergy testing demonstrated 100% synergistic activity for colistin–imipenem (FICI ≤0.5), whereas colistin–amikacin showed predominantly indifferent effects. *In vivo*, the combination of colistin–imipenem led to complete bacterial clearance from the mice with 100% survival, whereas the colistin–amikacin combination resulted in 100% mortality, similar to untreated controls.
Conclusions: The Colistin–imipenem combination demonstrates strong *in vitro* synergy and superior *in vivo* efficacy against colistin-resistant *P. aeruginosa*, highlighting its potential as a practical therapeutic option specially in resource-limited settings.

Keywords: Colistin resistance, *Pseudomonas aeruginosa*, MIC, Murine model

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium, widely distributed and causes nosocomial infections in immunocompromised patients and those in intensive care units. It is responsible for several infections, including ventilator-associated pneumonia (VAP), urinary tract infections (UTI), blood stream infections, surgical site

infections, wound infections (especially in burn sites) and chronic pulmonary infections in patients with cystic fibrosis.[1-3] It has an incredible ability to survive under numerous environmental conditions and has developed widespread resistance to last-resort antibiotics, which is now an alarming situation and a more concerning issue in global antimicrobial resistance (AMR) surveillance programs.[4] In low or middle-income countries, several factors, i.e., inconsistent antibiotic stewardship, inadequate infection prevention practices and limited laboratory diagnostic capacity, may be responsible for the dissemination of resistant strains of *Pseudomonas aeruginosa*.[5] Resistance to several antibiotics (β-lactams, aminoglycosides and fluoroquinolones) causing limitation of the treatment options, leading to prolonged hospital stays as well as higher mortality.[6] Colistin (polymyxin E) is widely regarded as a bactericidal, last resort antimicrobial drug for MDR Gram-negative bacteria such as *P.aeruginosa, Acinetobacter bumannii* and certain members of *Enterobacteriaceae*.[7,8] It binds to the lipid A component of the lipopolysaccharide of outer membrane of gram-negative bacteria, leading to kill the bacterial cells by membrane disruption with leakage of cellular gradients.[9]

However, the increasing use of colistin nowadays without appropriate indications is becoming an alarming emergence of colistin resistance across the globe. Colistin resistance is developed due to structural modification of lipid A, causing reduced colistin binding affinity. The addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) or phosphoethanolamine (pEtN) to lipid A is regulated by *PMR A/PMR B* AND *PHO P/PHO Q* (chromosomal mediated).[10,11] Plasmid-mediated genes (*mcr-1* to *mcr-10*) have been identified as colistin resistance genes, facilitating horizontal transmission of resistance, more in Enterobacteriaceae than *P. aeruginosa*.[12] Colistin-resistant *P. aeruginosa* is often associated with MDR or XDR resistance; hence, a combination of antimicrobial therapy like colistin with carbapenems or aminoglycosides becomes a more common way to improve antibacterial efficacy and antimicrobial coverage as well as to reduce the toxicity by lowering drug dosages and suppress the emergence of further resistance.[13] Other studies have established the synergistic or additive effects of colistin combined with carbapenems against MDR *P. aeruginosa*.[14,15]

For the evaluation of the therapeutic effectiveness and safety issues of the drug, *in vivo* studies are of equal importance as *in vitro* studies. Several factors, such as the pharmacokinetics of the drug, tissue penetration capacity, immune responses, and bacterial virulence, can significantly modulate the treatment outcomes *in vivo*.[16] Consequently, animal models, particularly murine infection models, play a crucial role in bridging the gap between laboratory findings and real-world therapeutic effectiveness.[17] In Bangladesh, data on systemic evaluation with molecular study and *in vitro* synergy testing with *in vivo* validation on colistin resistance in *P. aeruginosa* are so limited. This research aimed to evaluate the prevalence and molecular mechanism of colistin resistance in *P. aeruginosa*, *in vitro* and *in vivo* synergistic effects of colistin-based combinations, and to confirm their therapeutic effects, which may give evidence-based guidance for clinicians in resource-limited settings.

METHODS

Study design and clinical specimens

This cross-sectional experimental study was conducted in the Department of Microbiology, Dhaka Medical College Hospital (DMCH), Bangladesh, in 2019 from January to December. Study participants were recruited from the outpatient department and ICU of Dhaka Medical College Hospital (DMCH) irrespective of sex and antibiotic intake. A total of 350 clinical specimens were collected from adult patients (≥18 years), including wound swab, urine, endotracheal aspirates and blood samples, using strict aseptic techniques. Patients who were unwilling to give written consent were excluded from the study.

Isolation and identification of pseudomonas aeruginosa and antimicrobial susceptibility testing

MacConkey agar and blood agar were used to culture the specimens and incubated aerobically at 37°C for 18-24 hours. Based on colony morphology, non-lactose fermentation, pyocyanin or pyoverdine pigment production, grape-like odor, and metallic sheen, presumptive identification of *Pseudomonas aeruginosa* was done and confirmed by using standard biochemical tests; gram staining (gram-negative rods), oxidase and catalase positivity, growth at 42 °C, alkaline reaction on triple sugar iron agar, and oxidative utilization of glucose.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar following CLSI 2019 guidelines, using piperacillin–tazobactam (PIT), ceftazidime, cefepime, amikacin, ciprofloxacin, imipenem, aztreonam, AMC (Amoxycillin-Clavulanic acid), tigecycline, and colistin (screening only; MIC required for confirmation) according to the CLSI guideline.[18] Escherichia coli ATCC 25922 was used as the quality control strain.

Minimum inhibitory concentration determination

Minimum inhibitory concentrations (MIC) of colistin, imipenem, amikacin and piperacillin-tazobactam were determined by the agar dilution method according to CLSI 2019 guidelines.[18] Serial two-fold dilutions were done in Mueller-Hinton agar for each antibiotic. Agar plates were incubated at 37˚C for 18-24 hours after application of a standardized bacterial inoculum equivalent to 0.5 McFarland was applied on agar plates and a standardized bacterial inoculum equivalent to 0.5 McFarland. The lowest concentration of antibiotic inhibiting visible growth on agar media was considered as MIC of the drug of that

strain of bacteria.[18] Colistin resistance was defined as MIC ≥4 µg/ml. MIC was further confirmed by PCR.

Molecular detection of colistin resistance genes

Genomic DNA was extracted by using the boiling lysis method, and the selected isolates were re-extracted using a commercial DNA extraction kit for sequencing. Chromosomal colistin-resistant genes (*PMR A, PMR B, PMR C, PHO P* AND *PHO Q*) and plasmid-mediated mcr genes (*mcr-1* to *mcr-5*) were detected by PCR by following published protocols.[19,20] 1.5% agarose gel electrophoresis was used to select PCR products and visualized under UV using a gel documentation system. Amplified PCR products were purified (FAVORGEN Biotech Crop) and capillary sequenced (ABI 3500) and sequences were compared using BLAST against the GenBank database.[21]

In vitro synergy testing

In vitro synergy testing was conducted for two antibiotic combinations: colistin-imipenem and colistin-amikacin. For evaluating the interaction, agar dilution and fractional inhibitory concentration index (FICI) methods were used. FICI values were interpreted as synergy (≤0.5), additivity (>0.5-1), indifference (>1-4) and antagonism (>4). Synergy was defined when the MIC ≥4-fold decrease of both antibiotics compared with their MICs alone.[22]

In vivo animal study

The murine infection protocol was adapted from previously published animal infection models used to evaluate antimicrobial treatment in Gram-negative pathogens, including *Klebsiella pneumoniae* and *Acinetobacter baumannii*, with modifications for *P. aeruginosa*.[23,24]

Animal selection and infection model

To evaluate the effectiveness of the different antibiotic regimens, about 35 healthy Swiss albino mice (15-20 gm), irrespective of sex were obtained from ICDDR, B and acclimatized for seven days before the experiment by following international guidelines for animal care and use. A colistin-resistant *P. aeruginosa* isolate was sub cultured and adjusted to 0.5 McFarland (~10^4 CFU/ml) and mice were infected intraperitoneally with 250 µl of the suspension.[23]

Antibacterial treatment and microbiological assessments

35 mice were randomly divided into 7 groups (5 mice in each group). Among them, 6 groups were infected and 1 group was uninfected. The only uninfected group was the Negative Control group. Among the 6 infected groups, the only untreated group was the positive control group. The remaining 5 infected groups received antibiotic treatment. 3 individual groups received imipenem (60 mg/kg/day), colistin (3.4 mg/kg/day) and amikacin (15 mg/kg/day) alone, whereas the remaining 2 groups got colistin-imipenem and colistin–amikacin combinations at the same doses. All drugs were given intraperitoneally twice daily for 72 hours, which started after 4 hours of infection. Follow-up was given every 12 hours and survival was recorded. After 72 hours, mice were euthanized, blood was collected by cardiac puncture, incubated in tryptic soya broth (37˚C, 24 hours), then plated on blood agar and MacConkey agar to check bacterial clearance.[24]

Data analysis

All results were recorded systematically and analyzed using Microsoft Excel.

RESULTS

In vitro results

Among 350 clinical samples, 220 (63%) samples were obtained from male and 130 (37%) samples were obtained from female (Figure 1). Among these 350 samples, 236 (67.43%) samples yielded positive cultures. Among the 236 positive cultures, 63 isolates (26.7%) of *Pseudomonas aeruginosa* were detected, most commonly from wound swabs. Agar dilution MIC testing identified 12/63 (19.05%) isolates as colistin resistant, with all showing MIC ≥4 µg/ml. These colistin-resistant isolates showed high resistance to ciprofloxacin (85.33%), amikacin (75.00%), and imipenem (58.33%), while lower resistance was observed to piperacillin–tazobactam (25.00%) and tigecycline (41.67%). Overall, the remaining therapeutic options were limited for these isolates.

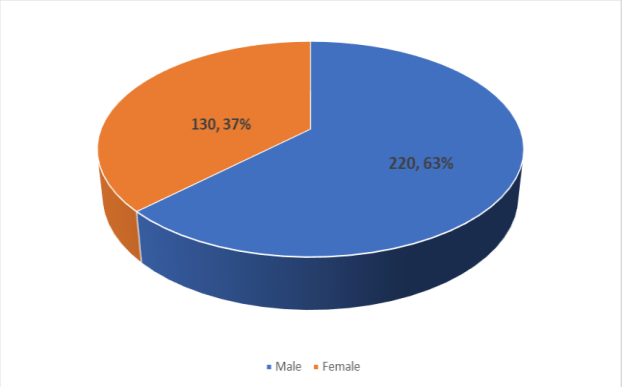


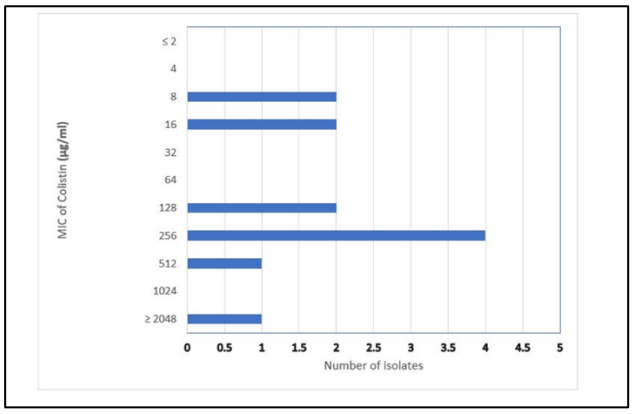
Figure 1: The distribution of samples according to gender (n=350).

Molecular analysis indicated chromosomal mechanisms of colistin resistance. Lipid A modification–associated genes within the *PMR* and *PHO* regulatory systems were frequent: *PMR C* and *PHO P* were each present in 6/12 (50.00%) isolates, *PMR B* and *PHO Q* in 3/12 (25.00%) isolates, and *PMR A* in 2/12 (16.67%) isolates (Table 1). Plasmid-mediated colistin resistance genes (*Mcr-1* to *mcr-5*) were not detected. No genes were detected in endotracheal aspirate or blood isolates.

Table 1: Distribution of colistin resistance–associated genes in *pseudomonas aeruginosa* isolates (n=12).

Gene	Wound swab N (%)	Urine N (%)	Total N (%)
PMR A	2 (16.67)	0 (0.00)	2 (16.67)
PMR B	3 (25.00)	0 (0.00)	3 (25.00)
PMR C	5 (41.67)	1 (8.33)	6 (50.00)
PHO P	5 (41.67)	1 (8.33)	6 (50.00)
PHO Q	2 (16.67)	1 (8.33)	3 (25.00)
Mcr-1 to Mcr-5	0 (0.00)	0 (0.00)	0 (0.00)

Colistin MICs among the 12 resistant isolates were heterogeneous (Figure 2). The most common MIC was 256 µg/ml (4/12, 33.33%), followed by 128 µg/ml (2/12, 16.67%); MICs of 16 and 8 µg/ml were each observed in 2/12 (16.67%) isolates. No isolate showed result within the susceptible range (≤2 µg/ml).



MIC=Minimum Inhibitory Concentration.

Figure 2: MIC of colistin among colistin-resistant isolates of *p. aeruginosa* (n=12).

In vitro synergy testing (n=4) showed clear differences between the combinations. Colistin–imipenem produced MIC reductions in all isolates (two 8-fold and two 4-fold reductions) with 100% synergism and low FICI values (0.25-0.50) (Tables 2-3). In contrast, colistin–amikacin demonstrated markedly limited activity, with only one isolate showing a 2-fold MIC reduction; overall, one isolate was additive (FICI=1) and three were indifferent (FICI=2).

In vivo therapeutic efficacy

Table 4 shows the *In vivo* therapeutic efficacy of antibiotic therapy on the clearance of colistin resistant *Pseudomonas aeruginosa* from blood among different groups of mice. All the mice in the positive control group were bacteraemic, whereas all mice in the negative control group were blood culture negative. Each of the 3 infected groups showed 80% were culture negative result with partial bacterial clearance, those who were treated with imipenem, colistin and amikacin as monotherapy. The infected group treated with colistin plus imipenem combination showed complete protection by 100% bacterial clearance, compared to uninfected control group. In contrast, treatment group with colistin plus amikacin combination resulted in 100% positive culture rate with no bacterial clearance.

Table 2: *In-vitro* efficacy and interaction outcome of colistin combinations against resistant *p. aeruginosa* (n=4).

Parameter	Cl+IMP, N (%)	Cl+AK, N (%)
Reduction of MIC (fold change)		
8-fold reduction	2 (50.00)	0 (0.00)
4-fold reduction	2 (50.00)*	0 (0.00)
2-fold reduction	0 (0.00)	1 (25.00)
No reduction (at baseline MIC)	0 (0.00)	3 (75.00)**
Interaction outcome		
Synergism	4 (100.00)	0 (0.00)
Additive	0 (0.00)	1 (25.00)
Indifference	0 (0.00)	3 (75.00)
Antagonism	0 (0.00)	0 (0.00)

* One *P. aeruginosa* isolate showing a 4-fold MIC reduction was susceptible to piperacillin-tazobactam.** One *P.aeruginosa* isolate showing no MIC reduction was susceptible to piperacillin-tazobactam. Abbreviations: CL=Colistin; IMP=Imipenem; AK=Amikacin.

Table 3: Comparison of efficacy of different antibiotic combinations by the FICI formula in colistin-resistant *pseudomonas aeruginosa*.

Antimicrobial combination	MIC value by agar dilution method (µg/ml)				FIC_a+ FIC_B	FICI	Effects
	Imipenem		**Colistin**				
	Alone	Combination	Alone	Combination			
Colistin+ imipenem	32	4	256	32	0.125+0.125	0.25	Synergistic
	32	4	512	64	0.125+0.125	0.25	Synergistic
	32	8	256	64	0.25+0.25	0.50	Synergistic
	32	8	128	32	0.25+0.25	0.50	Synergistic
	Colistin		**Amikacin**				
colistin+ amikacin	Alone	Combination	Alone	Combination			
	256	128	256	128	0.5+0.5	1	Additive

Continued.

Antimicrobial combination	MIC value by agar dilution method (µg/ml)				FIC_a+ FIC_B	FICI	Effects
	128	128	512	512	1+1	2	Indifference
	256	256	512	512	1+1	2	Indifference
	256	256	2048	2048	1+1	2	Indifference

Note: FICI=Fractional inhibitory concentration index. FICA=Fractional inhibitory concentration of one antibiotic in combination. FICB =Fractional inhibitory concentration of other antibiotic in combination. Synergy is defined as FICI ≤0.5 and/or ≥4-fold reduction in MIC. Additive effect defined as FICI >0.5–1 Indifference defined as FICI >1–4 MIC values were determined using the agar dilution method CL=Colistin; IMP=Imipenem; AK=Amikacin Interaction categories based on standard FICI criteria.

Finally, Colistin+Imipenem combination was considered as the most effective regimen, achieving complete bacteremial clearance. In contrast, the colistin with amikacin combination showed complete therapeutic failure, with persistent bacteremia, comparable to untreated controls.

Table 4: Results of antibiotic therapy on the clearance of *p. aeruginosa* from the blood of mice (n=35).

Groups (n=5/group)	Bacterial culture negative N (%)
Infected and untreated (positive control)	0 (0)
Uninfected (negative control)	5 (100)
Imipenem	4 (80)
Colistin	4 (80)
Amikacin	4 (80)
Colistin+imipenem	5 (100)
Colistin+amikacin	0 (0)
Total	22(62.86)

The combination of colistin with imipenem showed a 100% synergistic effect, while no synergism was observed in colistin with amikacin combination. The above finding indicates a strong correlation between the *in vivo* findings and the *in vitro* synergy results.

DISCUSSION

The present study provides an integrated approach to evaluate colistin-resistant *Pseudomonas aeruginosa* using phenotypic susceptibility testing, molecular analysis, *in vitro* synergy testing, and *in vivo* therapeutic validation. A combination of laboratory findings with an animal infection model ensures clinically relevant evidence to guide treatment strategies against colistin-resistant infections, especially in resource-limited settings. The isolation rate of *P. aeruginosa* observed in this study is consistent with its established role as a major nosocomial pathogen, especially in wound and respiratory tract infections. The detection of colistin resistance in a substantial proportion of isolates is concerning, given that colistin is frequently reserved as a last-line agent for infections caused by multidrug-resistant gram-negative bacteria. Similar increases in colistin resistance have been reported globally and are often attributed to selective antimicrobial pressure and limitations in antimicrobial stewardship practices.[9,25] In the present study, molecular analysis suggested the chromosomally mediated colistin resistance involving the *PMR* and *PHO* regulatory systems. The frequent detection of *PMR C* and *PHO P* genes is consistent with lipid A modification–associated resistance mechanisms previously reported in *P. aeruginosa*.[11] Importantly, no plasmid-mediated *mcr* genes were detected, suggesting that plasmid-mediated colistin resistance was not evident in this study. Together, these findings suggest that colistin resistance in the studied isolates was mainly associated with intrinsic chromosomal pathways under antimicrobial pressure.[12]

Combination therapy represents a rational approach to improve the therapeutic efficacy against colistin-resistant isolates. In this study, the *in vitro* synergy testing consistently showed superior activity by the colistin–imipenem combination. The marked reduction of MIC values and results of the fractional inhibitory concentration index displayed synergistic interactions by all the resistant strains of *P. aeruginosa*. These findings are similar to previous studies showing enhanced activity of polymyxin-carbapenem combinations against resistant *P. aeruginosa*. isolates.[14,15]

A key factor of this experimental study is the validation of *in vitro* findings using a murine infection model. The Colistin-imipenem combination resulted in 100% survival rate of the infected mice by ensuring complete bacterial clearance from them and thus showed therapeutic correlation with the observed synergy. The close association between *in vitro* synergy testing and *in vivo* outcomes validates the accuracy of synergy tests and acts as an indicator of therapeutic effectiveness. Another study also found a similar relationship between findings of laboratory synergy tests and experimental models of *P. aeruginosa* infection.[16]

From the clinical perspective, these findings are particularly significant for low- and middle-income countries like Bangladesh, where there is limited availability of newer antimicrobial agents. The relative availability of both colistin and imipenem ensures this combination as a suitable treatment option in such settings. The integrated approach of genetic resistance analysis, *in vitro* synergy testing and *in vivo* validation increases the translational relevance of this study and enables evidence-

based decision-making in managing colistin-resistant *P. aeruginosa* infections.

Limitations

The study was conducted at a single center with a limited number of colistin-resistant *P. aeruginosa* isolates. In addition, pharmacokinetic evaluation and assessment of other potentially effective antibiotic combinations were beyond the scope of this study.

CONCLUSION

This study revealed mainly the chromosomally mediated colistin resistance in *Pseudomonas aeruginosa*. Colistin–imipenem combination therapy showed a strong *in vitro* synergy and complete *in vivo* effectiveness. These findings highlight colistin–imipenem combination as a promising therapeutic strategy against colistin-resistant *P. aeruginosa*, particularly in resource-limited settings.

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REFERENCES

1. Tumbarello M, De Pascale G, Trecarichi EM, Spanu T, Antonicelli F, Maviglia R, et al. Clinical outcomes of Pseudomonas aeruginosa pneumonia in intensive care unit patients. Intensive Care Med. 2013;39(4):682-92.
2. Ramírez-Estrada S, Borgatta B, Rello J. Pseudomonas aeruginosa ventilator-associated pneumonia management. Infect Drug Resist. 2016;9:7-18.
3. Thaden JT, Park LP, Maskarinec SA, Ruffin F, Fowler VG, van Duin D. Results from a 13-Year Prospective Cohort Study Show Increased Mortality Associated with Bloodstream Infections Caused by Pseudomonas aeruginosa Compared to Other Bacteria. Antimicrob Agents Chemother. 2017;61(6):e02671-16.
4. Ali J, Rafiq QA, Ratcliffe E. Antimicrobial resistance mechanisms and potential synthetic treatments. Future Sci OA. 2018;4(4):FSO290.
5. Gandra S, Alvarez-Uria G, Turner P, Joshi J, Limmathurotsakul D, Van Doorn HR. Antimicrobial Resistance Surveillance in Low- and Middle-Income Countries: Progress and Challenges in Eight South Asian and Southeast Asian Countries. Clin Microbiol Rev. 2020;33(3):e00048-19.
6. Hirsch EB, Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.
7. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis Off Publ Infect Dis Soc Am. 2005;40(9):1333-41.
8. Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589-601.
9. Falagas ME, Rafailidis PI, Matthaiou DK. Resistance to polymyxins: Mechanisms, frequency and treatment options. Drug Resist Updat Rev Comment Antimicrob Anticancer Chemother. 2010;13(5):132-8.
10. Tamayo R, Choudhury B, Septer A, Merighi M, Carlson R, Gunn JS. Identification of cptA, a PmrA-regulated locus required for phosphoethanolamine modification of the Salmonella enterica serovar typhimurium lipopolysaccharide core. J Bacteriol. 2005;187(10):3391-9.
11. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014;5:643.
12. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161-8.
13. Abd El-Baky RM, Masoud SM, Mohamed DS, Waly NG, Shafik EA, Mohareb DA, et al. Prevalence and Some Possible Mechanisms of Colistin Resistance Among Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa. Infect Drug Resist. 2020;13:323-32.
14. Bergen PJ, Bulman ZP, Saju S, Bulitta JB, Landersdorfer C, Forrest A, et al. Polymyxin Combinations: Pharmacokinetics and Pharmacodynamics for Rationale Use. Pharmacotherapy. 2015;35(1):34-42.
15. Daoud Z, Mansour N, Masri K. Synergistic Combination of Carbapenems and Colistin against P. aeruginosa and A. baumannii. Open J Med Microbiol. 2013;3(4):253-8.
16. Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N. In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in Pseudomonas aeruginosa biofilm infection. Antimicrob Agents Chemother. 2012;56(5):2683-90.
17. Sarkar S, Heise MT. Mouse Models as Resources for Studying Infectious Diseases. Clin Ther. 2019;41(10):1912-22.
18. CLSI. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. CLSI supplement M100.

Wayne, PA: Clinical and Laboratory Standards Institute. 2019. Available at: https://scholar.google.com/scholar_lookup?title=Performance%20standards%20for%20antimicrobial%20susceptibility%20testing.%20CLSI%20supplement%20M100&publication_year=2019. Accessed on 11 March 2026.
19. Haeili M, Javani A, Moradi J, Jafari Z, Feizabadi MM, Babaei E. MgrB Alterations Mediate Colistin Resistance in Klebsiella pneumoniae Isolates from Iran. Front Microbiol. 2017;8:2470.
20. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Eurosurveillance. 2018;23(6):17-672.
21. Almira EC, Panayotova N, Farmerie WG. Capillary DNA sequencing: maximizing the sequence output. J Biomol Tech JBT. 2003;14(4):270-7.
22. Doern CD. When Does 2 Plus 2 Equal 5? A Review of Antimicrobial Synergy Testing. J Clin Microbiol. 2014;52(12):4124-8.
23. Toledo PV, Tuon FF, Bail L, Manente F, Arruda P, Aranha-Junior AA. Experimental model for treatment of extended-spectrum β-lactamase–producing Klebsiella pneumoniae. Arq Bras Cir Dig. 2014;27(3):168-171.
24. Rodríguez-Hernández MJ, Pachón J, Pichardo C, Cuberos L, Ibáñez-Martínez J, García-Curiel A, et al. Imipenem, doxycycline and amikacin in monotherapy and in combination in Acinetobacter baumannii experimental pneumonia. J Antimicrob Chemother. 2000;45(4):493-501.
25. Jeannot K, Bolard A, Plésiat P. Resistance to polymyxins in Gram-negative organisms. Int J Antimicrob Agents. 2017;49(5):526-35.

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