Original Research Article

Effectiveness of Polymyxin B in Multidrug Resistance Gram Negative Bacilli: A Cross-Sectional Study

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ABSTRACT

INTRODUCTION

Antibiotic resistance remains a global public health threat, posing serious treatment challenges, particularly for hospitalised patients. Owing to increasing resistance to most of the commonly prescribed antibiotics, a newer antibiotic or a re-emerge of an older class of drug will be mainstay of treatment against Multidrug Resistant (MDR) organisms. Carbapenems (such as meropenem, imipenem, and ertapenem,) have traditionally been the frontline antibiotics for severe multidrug-resistant (MDR) Gram-negative infections. With the rise of carbapenem-resistance, need for other effective alternate drugs is on the rise. Despite the reported toxicities, Polymyxin B is considered as a last resort drug for Multidrug-resistant gram-negative bacterial infections. The present study was aimed to determine the in vitro effectiveness of Polymyxin B in MDR gram negative bacterial isolates and compare its MIC with that of carbapenem class of antibiotics.

METHODS

A total of 130 non repetitive CRE gram negative bacterial isolates from various clinical specimens were identified by conventional methods and VITEK-2 ID system. The antibiotic susceptibility of these isolates was then determined by both disc diffusion and VITEK-2 AST. The MIC of test isolates to Polymyxin B (PB) was done by Epsilometery strip (E-strip) and compared with that of meropenem. Statistical analysis was calculated with Open Epi using two by two table version 3.01.

RESULTS

A total of 130-gram negative bacterial MDR isolates from clinical specimens; urine 76 (58.5%), pus 35 (27%), blood 11 (8.5%) and BAL 8 (6%) were included in the study. Out of these, 118 (94.4%; n=125, *Protea* group were excluded) were susceptible for polymyxin B. Susceptibility rate of MDR isolates of *Pseudomonas spp.* and *Klebsiella pneumoniae* was at 91.3 % *and* 87.5% respectively and that of MDR *Acinetobacter spp. showed* 75%. Escherichiae group (including *E. coli, Citrobacter spp.* and *Enterobacter spp.*) showed 100% susceptibility. 79 (94%) of the meropenem susceptible strains and 30 (73.1%) of the meropenem resistant strains showed susceptible to polymyxin B, which is statistically significant (p-value of <0.05).

CONCLUSION

Polymyxin B can be considered as an effective treatment option against MDR bacterial organisms and significantly better alternate to meropenem. Further in vitro studies will be required to evaluate polymyxin B against the newer antibiotics such as the fifth generation cephalosporins.

KEYWORDS: Multi Drug Resistance, E-Strip, Meropenem, Polymyxin B.

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INTRODUCTION

Antimicrobial resistance (AMR) is an ever-growing serious threat to global health compromising and undermining the effectiveness of life-saving treatments and adversely affecting the health care costs. “In 2023, approximately one in six laboratory-confirmed bacterial infections worldwide were caused by bacteria resistant to antibiotics”.[1]

Also, the global economical impact of AMR is highly concerning, with a WHO report stating that, by year 2050, the cost burden of AMR could reach $100 trillion if not addressed.[2]

Current trends in AMR indicate an increasing threat from Gram negative bacterial infections. which has worsened over the past few decades due to the increasing incidence of multi-drug-resistant organisms (MDROs) from both community- and hospital-acquired infections.[3]

Multidrug-resistant Gram-negative Bacteria (MRGN) are defined as exhibiting in-vitro non-susceptibility, intermediate sensitivity or resistance to at least one agent in 3 or more antimicrobial classes. within the antibacterial spectrum, which pose major treatment challenges, demanding the highest priority, particularly among the hospitalised patients due to the Hospital Acquired Infections (HAIs).[4,5]

Particularly in developing nations such as India, HAI and the associated multi drug resistance has been on the rise. Many centres have reported resistance to aminoglycosides ranging from 32.6-83.6% on an average and 41-80% rate of resistance to β-Lactams (BL) and β-lactamase Inhibitors (BL-BLI).[6,7] The quinolones resistance is approximately 30% whereas carbapenem resistance rate among Gram Negative Bacilli (GNB) is 36.4%.[8,9] Percentage of Extended Spectrum Beta Lactamases (ESBL) producing bacteria range from 66.8-71.5% and Colistin resistance rate is approximately reported at 20%.[10,11]

Carbapenems (such as meropenem, imipenem, and ertapenem,) have been the frontline antibiotics for managing severe multidrug-resistant (MDR) Gram-negative infections. However, with the emergence of carbapenem-resistant Enterobacteriaceae (CRE), the ensuing treatment challenges have raised demands for effective utilization of old antibiotics against such multidrug and extremely drug resistant (MDR and XDR) bacteria.[12-14]

Polymyxins which include polymyxin B and colistin (polymyxin E) are the “old” antibiotics which are often regarded as the last resort in MDR Gram-negative infections or salvage therapeutic option for carbapenem-resistant (CR) GNB.

These are polypeptide antibiotics with a unique chemical structure and a detergent-like effect on lipid structures that can effectively disrupt the outer membrane integrity of Gram-negative bacilli and therefore effective against wide range of multidrug-resistant and nosocomial bacteria.[14-17]

In addition to the increasing drug resistance along with a decline in the development of newer antibiotics has led to the re-introduction of polymyxins in clinical practice particularly for the treatment of MDR and XDR gram negative bacilli, despite reported toxicities (neurotoxicity and nephrotoxicity). Such increase in therapeutic use of polymyxins, have renewed the interest in their in-vitro and in-vivo susceptibilities.[17-20]

Unlike colistin, polymyxin B is administered in its active form and is excreted mainly by non-renal means. Thus, owing to better pharmacokinetics and lower risk of nephrotoxicity, polymyxin B (PMB) has been recommended for systemic use for treatment of invasive infections, over colistin (Polymyxin E).[20-22]

The increasing incidence of infections by multi drug resistant bacteria have drastically limited the treatment options with existing commonly prescribed antibiotics and therefore a newer drug or a re-emerge of an older class of antibiotic will be a choice in the treatment of these MDR organisms. In this pursue, the current study was undertaken to evaluate the effectiveness of Polymyxin b in MDR gram negative isolates by determining its Minimum Inhibitory Concentration (MIC) and to compare its effectiveness with that of carbapenems (meropenem).

MATERIALS AND METHODS

A cross-sectional periodical study was conducted between November 2024 to January 2026 in the Department of Microbiology, South India a tertiary care centre after obtaining the Institutional Ethics Committee (IEC) approval, REF: IEC/NMC/25/SEP24/19.

Study Procedure

A total of 130 non repetitive MDR gram negative bacterial isolates from clinical specimens of exudates, Broncho-Alveolar Lavage (BAL), urine and blood, were identified up to species level by conventional methods (Biochemical reactions like indole test, urease enzyme production, citrate utilisation. Triple sugar iron, mannitol fermentation and motility test, phenyl alanine deaminase test, oxidase test, catalase test etc.,) and/or automated system (VITEK-2 system (Vitek2 GN ID-card; BioMerieux, Brussels, Belgium) and were included in the study. [Table-1].

Antibiotic susceptibility testing of the isolates for ampicillin (10 µg), cephelexin (30 µg), cefotaxime (30 µg), cephalexin (30 µg), trimethoprim-sulfamethoxazole (1.25 µg and 23.75 µg), cefepime (30 µg), amikacin (30 µg), cefaperazone sulbactam (15/10 µg), piperacillin tazobactum (100 µg and 10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg) and tobramycin (10 µg) was determined by both disc diffusion and VITEK 2 system (vitek2 GN AST cards 405,406 and 477 for urine; BioMerieux, Brussels,

Belgium) and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2026.[23]

MIC of 125 study isolates (*Protea* group were excluded) to polymyxin B (PB) was done by Epsilometery strip (E-strip) obtained from HiMedia, Mumbai and interpreted as per CLSI guide lines 2026 [Table-2].[23] The MIC breakpoints of PB as follows; MIC < 2 as Sensitive, MIC 4 mg/L as Intermediate and > 8 mg/L as resistance. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Procured from HiMedia) were used as controls and the controls were satisfactory.

Statistical analysis was calculated with Open Epi using two by two table version 3.01. The p-value was calculated by Chi-square test and p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 130-gram negative bacterial isolates screened as MDR from various clinical specimens, urine 76 (58.5%), pus 35 (27%), blood 11 (8.5%) and BAL 8 (6%), were included in this study. Out of these 130 samples, 77 were isolated from males and 53 from females (M:F ratio=1.4:1). The mean age of the study population was 44.3 years.

Bacterial isolates break up from the various clinical samples is shown in the [Table 1] and antibiotic susceptibility percentage of the study isolates for the routine antibiotics is shown in the [Table 2].

Organisms	Urine n (%)	Pus n (%)	Blood n (%)	Bal n (%)	Total n (%)
Escherichia coli	40 (53)	13 (37)	5 (45.4)	3 (37.5)	61 (47)
Klebsiella pneumoniae	18 (24)	9 (26)	3 (27.3)	2 (25)	32 (24.5)
Pseudomonas spp.	8 (10)	11 (31.4)	3 (27.3)	1 (12.5)	23 (18)
Acinetobacter spp.	2 (2.6)	0	0	2 (25)	4 (3)
Enterobacter spp.	2 (2.6)	1 (2.8)	0	0	3(2.3)
Citrobacter spp.	1 (1.3)	1 (2.8)	0	0	2 (1.5)
Proteus spp.	2(2.6)	0	0	0	2 (1.5)
Providencia spp.	2(2.6)	0	0	0	2(1.5)
Morganella spp.	1 (1.3)	0	0	0	1 (0.7)
Total	76	35	11	8	130

Table 1: Specimen wise distribution of the bacterial isolates

Antibiotics	Sensitive (%)	Resistant (%)	Total isolates
Ampicillin	-	100	130
Cephalexin	-	100	130
Cefuroxime	-	100	51
Cephotaxim	-	100	130
Ceftazidime	-	100	80
Cefepime	11 (8.5)	119 (91.5)	130
Cefaperazone sulbactum	52 (40)	78 (60)	130
Piperacillin tazobactum	52 (40)	78 (60)	130
Amikacin	94 (72)	36 (28)	130
Tobramycin	94 (72)	36 (28)	130
Ciprofloxacin	11 (19.3)	46 (80.7)	57
Ofloxacin	25 (19)	105 (81)	130
Imepenem	87 (67)	43 (33)	130
Meropenem	87 (67)	43 (33)	130

Table 2: Antibiotic susceptibility results of the isolates

All the test isolates were subjected for MIC determination by Epsilometry test (E-strip) for Polymyxin B (PB). Susceptible percentage of test isolates were as follows:

A total of 118 (94.4%; n=125, *Protea* group were excluded) were susceptible for polymyxin B, out of which 7 (5.9%) isolates were of intermediate susceptibility.

Among the MDR gram negative bacilli, *Escherichia coli* showed the highest susceptibility rate of 100% along with *Enterobacter* and *Citrobacter* species; followed by *Pseudomonas spp.* (91.3% susceptibility) and *Klebsiella pneumoniae* (87.5% susceptibility) with *Acinetobacter spp.* showing the least susceptibility (75%) to PMB. [Table-3].

Isolates	Susceptible			Resistant	Total isolates
	Sensitive (<2µg/ml)	Intermediate (4µg/ml)	Total (%)	> 8 µg/ml	(%)
Escherichia coli	61	0	61 (100%)	0	61 (48.8)
Klebsiella pneumoniae	24	4	28 (87.5%)	4 (12.5%)	32 (25.6)
Pseudomonas spp.	19	2	21 (91.3%)	2(8.7)	23 (18.4)
Acinetobacter spp.	2	1	3 (75%)	1 (25%)	4 (3.2)

Enterobacter spp.	3	0	3 (100%)	-	3 (2.4)
Citrobacter spp.	2	0	2 (100%)	-	2 (1.6)
Total	111	7	118 (94.6 %)	7 (5.4 %)	125

Table 3: Polymyxin B susceptibility for MDR isolates by E-strip

Polymyxin B susceptibility and its statistical significance: The comparison of susceptibility of polymyxin B and meropenem is shown in [Table-4/Fig-1].

Among the 84 meropenem susceptible strains (n=125, *Protea* group were excluded), 79 (94%) showed susceptible to polymyxin B with statistically significant p-value <0.05. A total of 30 (73.1%) out of 41 meropenem resistant strains were susceptible to polymyxin B with statistically significant p-value of <0.05.

Drug	**Susceptible n (%)**	**Resistant n (%)**	**Total**
Meropenem	84 (67.2%)	41 (32.8%)	125
Polymyxin B	118 (94.4%)	7 (5.6)	125
Drug	**Polymyxin B**		
	Susceptible n (%)	Resistant n (%)	Total
Meropenem susceptible	79 (94 %)	5 (6%)	84
Meropenem resistant	30 (73.1%)	11 (26.8%)	41
Total	**109**	**16**	**125**

Table 4: The comparison of susceptibility of Polymyxin B and Meropenem for MDR gram negative bacilli

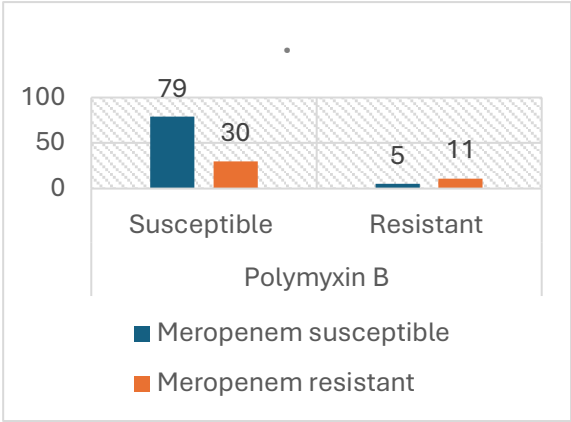

Figure 1: The comparison of susceptibility of Polymyxin B and Meropenem for MDR gram negative bacilli

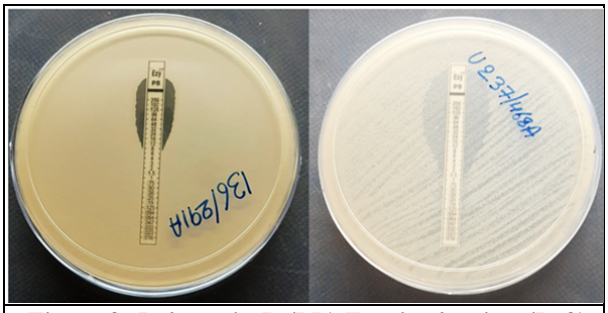

Figure 2: Polymyxin B (PB) E-strip showing (Left) and Susceptible (Right) to the test isolate

DISCUSSION

In the current era of MDR and XDR bacterial infections, particularly in hospitalised patients and with limited availability of newer drugs, Polymyxin B (PMB) has re-emerged as an effective alternate for treatment of multidrug resistant gram-negative bacterial infections.

Zhang *et al.*, in his multicenter study observed a positive clinical and safety outcome for PMB in the treatment of carbapenem-resistant Gram-negative bacterial infections.[20]

Similarly, Yan A et al. in their retrospective case series and literature review in 2025, demonstrated favourable clinical responses and pathogen clearance rates in the paediatric age group.[21]

In an in-vitro study by Thamlikitkul et al., against carbapenem-resistant *Acinetobacter baumannii,* 98.2% susceptibility to polymyxin B was reported.[24].

These finding are similar to a Mexican study, wherein Rosales-Reyes et al. demonstrated 100% susceptibility of highly lethal and biofilm producing clone (92.9% strains) of MDR *A. baumannii*, to polymyxin B.[25]

Bratu S et al. in their independent studies, reported a 90% in vitro susceptibility rate for polymyxin b against carbapenem resistant *Klebsiella pneumoniae*

and a 100% susceptibility rate against multidrug-resistant strains of *Pseudomonas aeruginosa.*[26,27]

In the present study also, similar findings were observed with 91.3 % and 87.5 % susceptibility to *Pseudomonas spp.* and *K. pneumoniae* respectively and 75% with *Acinetobacter baumannii* isolates.

In the comparative susceptibility of polymyxin B and Meropenem, the present study showed a statistically significant higher susceptibility rate for polymyxin B (94% of meropenem susceptible strains and 73.1% of meropenem resistant strains), as compared to meropenem.

Several studies have demonstrated that the combinations of polymyxin B and various traditional antibiotics result in increased antibacterial efficacy, highlighting the potential of combination therapy in addressing drug-resistant bacteria.[14]

Barth et al. in their evaluated activity of polymyxin B in combination with imipenem, meropenem, or tigecycline in KPC-2 producing Enterobacteriaceae and observed that Polymyxin B plus carbapenem combination was most effective against *K. pneumoniae* and *Enterobacter cloacae* compared to tigecycline combination.[28]

In another study, Tian et al. in his study in 2021 indicated that combination of polymyxin B and tigecycline as promising strategy[29] and similarly, Rahim et al. reported synergistic efficacy of polymyxin B and chloramphenicol in MDR NDM-producing *Klebsiella pneumoniae*.[30]

However, in a meta-analysis by Chen J et al. in 2024, the comparison of polymyxins and Ceftazidime–avibactam (CEF-AVI) in treatment of CRE infections, it was concluded that CEF-AVI showed better efficacy with lower mortality rate and higher microbial clearance rate.[31]

In Conclusion, Polymyxin B can be an effective alternate therapeutic option against multi-drug and extensive drug-resistant gram-negative bacilli with significant higher in-vitro susceptibility rate as compared to meropenem, which is of higher relevance in consideration of the increasing incidence of carbapenem resistant strains of MDR bacteria. However, further prospective studies are needed to determine the microbiological effectiveness of PMB against other newer antibiotics and combinations such as Ceftazidime–avibactam and Cefiderocol.

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