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The in vitro activity of Polymyxin B against Multi drug resistant bacterial isolates

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Abstract

There has been a resurgence in the use polymyxins for the treatment of infections due to multi drug resistant (MDR) gram negative bacilli but unfortunately even polymyxins with time can develop resistance. This has necessitated the need to know their susceptibility trends. This study was conducted to investigate the in vitro activity of polymyxin B against a collection of MDR gram negative isolates from our hospital. A prospective, hospital based study was conducted for a year in which all the gram negative isolates obtained from clinical samples, were subjected to the testing of their antimicrobial susceptibilities to different groups of drugs including polymyxin B by Kirby-Bauer disk diffusion method. Based on this, the isolates which were labelled as MDR were further subjected to the Epsilometer test (E-test) and broth micro dilution (BMD) to determine the minimum inhibitory concentrations (MIC) of polymyxin B. Out of the 120 MDR isolates, 6 (5%) were resistant to polymyxin B by the BMD method which is the reference method. Of the six isolates resistant by BMD, one *Klebsiella pneumoniae* isolate was found sensitive by disc diffusion method (very major error). One hundred fourteen isolates were sensitive by all the three methods. The rates of major errors for E-test and disc diffusion method were 0%. Therefore polymyxin B is an effective drug against MDR gram negative isolates but its use should be cautious and based on the results of standard susceptibility method like BMD.

Keywords: Polymyxin B, Multi drug resistant, carbapenems, MDR.

Introduction

Rising antimicrobial resistance and emergence of multi drug resistant (MDR) bacteria worldwide is one of the major health problems facing the world today[1]. Worsening the scenario is paucity of new antimicrobials which has led to resurgence of polymyxins and established there use in the treatment of infections due to MDR gram negative bacilli especially those that are resistant to carbapenems [2, 3].Polymyxins are a group of five different polypeptide antibiotics (polymyxins A, B, C, D, and E), out of which only polymyxin B and polymyxin E are in clinical use and act primarily on the gram-negative bacterial cell wall. These drugs cause rapid permeability changes in the cytoplasmic membrane leading to cell death [3, 4]. Unfortunately even polymyxins with time can develop resistance due to changes in the outer membrane due to loss of LPS or/and PmrAB two component system [5]. Thus, taking into account increase in clinical use of polymyxins

as the last resort drug for the treatment of life-threatening gram negative infections, has necessitated the need for developing and knowing their susceptibility trends. With this background this study was conducted to investigate the in vitro activity of polymyxin B against a collection of MDR gram negative isolates at our hospital.

Material and Method

A hospital based prospective study was done on MDR gram negative bacterial isolates obtained from samples received in the bacteriology lab of our hospital after approval from institutional ethical committee, for a period of one year from January 2018 to December 2018. The isolates were identified by conventional biochemical methods according to standard microbiological techniques [6]. Antibiotic Susceptibility Testing was done on Mueller-Hinton agar by Kirby Bauer's disc diffusion method, and the results were interpreted according to the CLSI guidelines, 2016 [7]. The following antimicrobial discs (µg) were used: cefotaxime (30), cefepime(30), ceftazidime (30), ceftriaxone (30), gentamicin (10), amikacin (30), tobramycin (10), ciprofloxacin (5), levofloxacin (10), co-trimoxazole (25), imipenem (10), piperacillin and tazobactam (100+10), cefoperazone and sulbactam (75+30), tigecycline (15), colistin (10) & polymyxin-B (300U) .The isolates were labeled as MDR by being resistant to at least one agent among three or more antimicrobial classes as recommended by European Centre for Disease Prevention and Control [8].

Minimum inhibitory concentration testing (MIC)

The MIC of polymyxin B for all the MDR gram negative bacterial isolates was determined by the E-test strips according to the manufacturer's instructions & Broth micro dilution (BMD) by non automated method in polystyrene plates with cation-adjusted Mueller–Hinton broth [9]. The MIC by E test strip was read at the intersection of inhibited growth and was rounded up to the next highest two-fold dilution [10]. In BMD method MIC was considered as the lowest concentration of polymyxin B at which no visible growth was obtained. MIC breakpoints (µg/ml) of polymyxin B for *Pseudomonas aeruginosa* (≤ 2 Susceptible, 4 intermediate, ≥ 8 resistant) and *Acinetobacter* (≤ 2 Susceptible, ≥ 4 resistant) were used as per CLSI guidelines. For *Enterobacteriaceae* EUCAST MIC breakpoint (≤ 2 Susceptible, > 2 resistant) for colistin was used [11]. *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 were used as controls. The performance of E-test & disk diffusion was compared with the reference method i.e BMD. The MICs were considered in essential agreement if they were ±1 twofold dilution and in categorical agreement if the results were in the same interpretive category. Very major error was labelled when result was false-susceptible by the disk diffusion/E-test; major error when false-resistant result were produced by the disk diffusion/E-test. Unacceptable levels were taken as ≥1.5% for very major errors and ≥ 3% for major errors [12, 13].

Results

Out of a total of 5000 samples received during the study period, 120 MDR isolates were obtained and included in the study. The most common MDR pathogen among the isolates were *Pseudomonas aeruginosa.* followed by *Klebsiella pneumoniae.* Catheters followed by urine samples from intensive care unit were the common sources of MDR isolates (Table 1).

Table 1: Source wise distribution of the MDR isolates included in the study

Isolate	Source					Total
	Catheter tips	Urine	Pus	Blood	BAL	
P.aeruginosa	18	5	6	2	7	38
K.pneumoniae	12	7	4	5	4	32
A.baumannii	3	5	8	8	3	27
E.coli	7	8	2	1	-	18
Enterobacter	-	3	-	2	-	5
Total	40	28	20	18	14	120

Highest sensitivity of the MDR isolates included in the study were to polymyxin B (95.8%) followed by tigecycline (85.8%) and imipenem (78.3%). Four *Pseudomonas aeruginosa* Isolates and 1 *Acinetobacter baumannii* isolate were found to be resistant to Polymyxin B by the disc diffusion method (Table 2).

Table 2: Antimicrobial resistance pattern of the MDR isolates by disc diffusion method

Antimicrobial agent	*P.aeruginosa* n(%)	*pneumoniae* n (%)	*A.baumannii* n (%)	*E.coli* n(%)	*Enterobacter* n(%)
Cefotaxime	26 (68.4%)	23 (72%)	24 (88.9%)	13 (72.2)	3(60)
Cefepime	25 (65.7%)	24 (75%)	25 (92.5%)	12 (66.7)	4(80)
Ceftazidime	26 (68.4%)	24 (75%)	24 (88.9%)	14 (77.8)	3(60)
Ceftriaxone	27 (71.1%)	28 (87.5%)	25 (92.5%)	14 (77.8)	4(80)
Gentamycin	30 (78.9%)	18 (56.2%)	22 (81.5%)	10 (55.5)	2(40)
Amikacin	28 (73.7%)	19 (59.4%)	25 (92.5)	6 (33.3)	3(60)
Tobramycin	28 (73.7%)	ND	ND	ND	ND
Ciprofloxacin	34 (89.5%)	26 (81.2%)	24 (88.9)	16 (88.9)	4(80)
Levofloxacin	35 (92.1%)	25 (78.1%)	25 (92.5)	15 (83.3)	4(80)
Imipenem	6 (15.8%)	6 (18.7%)	12 (44.4)	1 (5.5)	1(20)
Tigecycline	14 (36.8%)	2 (6.2%)	1 (3.7)	0	0
Piperacillin/ Tazobactam	21 (55.3%)	16 (50%)	14 (51.8)	5 (27.8)	2(40)
Cefoperazone/ Sulbactam	23 (60.5%)	17 (53.1%)	15 (55.5)	4 (22.2)	2(40)
Polymyxin-B	4 (10.5%)	0	1 (3.7)	0	0

n* -No. of isolates resistant to particular antimicrobial

Out of the 120 MDR isolates 6 (5%) were resistant to polymyxin B by the BMD method which is the reference method. Of the six isolates resistant by BMD, one *Klebsiella pneumoniae* isolate was found sensitive by disc diffusion method (very major error). One hundred fourteen isolates were sensitive by all the three methods. The rates of major errors for E-test and disc diffusion method were 0% (Table 3)

Table 3: Comparison of disk diffusion, E-test & BMD in Polymyxin B-resistant bacteria

Isolate	BMD (µg/ml)	E-test (µg/ml)	Disk diffusion (mm)
Pseudomonas aeruginosa	256	256	0
Pseudomonas aeruginosa	256	128	0
Pseudomonas aeruginosa	128	64	0
Pseudomonas aeruginosa	64	16	0
Klebsiella pneumoniae	16	8	15
Acinetobacter baumannii	512	>256	0

Discussion

MDR isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* sensitive only to polymyxins have emerged as an important cause of healthcare associated infections especially in patients admitted in the intensive care units [14, 15, 16, 17]. Therefore polymyxin group of drugs have emerged as a last resort for the treatment of life-threatening infections and there is a pressing need to update the susceptibility data in every setup.

In our study an overall high susceptibility rate of 95% to polymyxin B was seen among MDR Isolates, whereas 91.5% sensitivity was reported from New Delhi and 100% sensitivity has been reported from Egypt [3, 18]. Amongst the MDR isolates *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* showed 89.5%, 96.9% and 96.3% sensitivity to polymyxin B respectively. Some workers have reported 100% sensitivity to polymyxin B amongst *Pseudomonas spp.* & *Acinetobacter baumannii isolates* [14, 15]. High resistance to polymyxin B amongst *Klebsiella pneumoniae* & *Acinetobacter baumannii* has been reported from Brazil & Rio de Janeiro [17, 19].

The 114 MDR isolates sensitive to polymyxin B were correctly identified by all the three methods disc diffusion, E-test, BMD in our study. Out of the six isolates resistant to polymyxin B, one was identified as sensitive by the disc diffusion method (very major error: 0.8%) but there were no major errors. The results are comparable to that of Behera et al who reported 1 % very major error with polymyxin B disc diffusion method and no major errors [3]. Although no discrepancy was seen between MIC by E-test and BMD in our study but many reports have reported errors in E-test and questioned its reliability [3, 20].

Our study demonstrated a good sensitivity of various MDR gram negative isolates to polymyxin B and therefore rationalizes its use and resurgence in treatment of such infections. Empirical use of polymyxin B should be avoided to limit the emergence of resistance among gram negative bacilli. Clinical use must be supported by a reliable & validated in vitro susceptibility test results. The disk diffusion and E-test methods although are convenient and appealing for use in busy laboratories but should not be used as there can be very major errors in these tests. So, BMD is the recommended method for MIC testing.

Conclusion

Although the antimicrobial activity of polymyxin B remains high in many of the centres as of now but emergence of even few polymyxin B resistant isolates is worrisome. So the use should be cautious and based on the results of standard susceptibility method like BMD and also future studies should be planned on evaluating appropriate combination therapies for its optimal use.

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