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Original Research Article

Evaluation of MIC Colistin in Metallo-beta-lactamase producing Gram negative bacilli by Broth microdilution

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ABSTRACT

Introduction: Carbapenemase producing Enterobacterales and nonfermenter organisms like *Pseudomonas* aeruginosa and *Acinetobacter* sp. cause difficult to treat life treating infections. Colistin plays important role in treating such infection. Broth microdilution is recommended by EUCAST and CLSI for MIC determination of colistin.

Materials and Methods: Gram negative bacilli resistant to Imipenem were subjected to test for Metallo-beta-lactamase (MBL) production by Disc potentiation test. MIC determination of colistin was done by Broth microdilution (BMD) in MBL producing isolates.

Result: 20.5% of isolates of *Ecoli*, *Klebsiella* sp., *Pseudomonas* and *Acinetobacter* sp. were found to be MBL producers. All *Ecoli* were sensitive to colistin. 1 isolate of *Pseudomonas* sp. and *Klebsiella* sp. each were found to be resistant. 3 isolates of Acinetobacter sp. were resistant to colistin.

Conclusion: MBL producing Enterobacterales and nonfermentors like *Pseudomonas* sp. showed good sensitivity to colistin. *Acinetobacter* sp. showed 17.64% resistance to colistin.

1. Introduction

Gram negative bacteria are important cause of serious hospital acquired infection in admitted patients. Multiple Beta lactamase enzymes produce by these organisms make the treatment of these bacteria more complicated.[1,2]

Carbapenem resistant Enterobacterales (CRE), which are characterized by rapid and progressive dissemination are important cause of nosocomial infection around the world.[2] Production of betalactamase enzymes that can hydrolyse carbapenems is one of the main mechanisms of resistance in Enterobacterales. Carbapenemases can be classified into molecular class A (*Klebsiella pneumoniae* C -KPC), Class B (Metallo-beta-lactamase) & class D Oxa 48.[2,3]

Carbapenemase gene transfer is plasmid mediated which results in involvement of multiple pathogens and become widespread in hospital settings.[2] Nonfermenter organism like *Acinetobacter* & *Pseudomonas* may become resistant to carbapenems by different mechanisms other than carbapenemase production such as decreased permeability, altered penicillin binding protein and sometimes efflux pump overexpression.[3]

Colistin is one of the last few options for the treatment of drug resistant Gram-negative bacteria. For difficult to treat Gram negative 'Super bugs', Polymyxins are a critically important component.[4] Polymyxins are polypeptide antibiotics, which are cationic in nature and colistin is a member of this group. Resistance of Colistin ought to be monitored as it is increasingly used in treating infections caused by multi-drug resistant bacteria. Although there are different ways to detect the colistin resistance in bacterial strains, the Broth Microdilution (BMD) is recommended method for detection of colistin resistance in bacteria by the EUCAST- CLSI Polymyxin Breakpoints Working Group.[5] This study was therefore conducted

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to monitor colistin resistance in Metallo-beta-lactamase producing Gram negative bacilli in our hospital.

2. Materials and Methods

We conducted this cross-sectional study in a tertiary care hospital from 2018 to 2019 in Department of Microbiology. It is approved by the Institutional Ethics Committee. All culture samples received routinely in the department were processed by conventional method. Gram negative rods were identified and subjected to antibiotic susceptibility testing. All consecutive nonduplicate Gram negative bacilli resistant to Imipenem were subjected to Metallo-beta-lactamase (MBL) detection by disc potentiation test using Imipenem (10ug) and Imipenem (10ug) + EDTA (750ug). Increase in the Zone size of >= 7 mm was considered MBL producer.[5,6] All Metallo-beta-lactamase producing gram negative bacilli were included in the study. Other Carbapenemase producing gram negative bacilli were not included in the present study.

MIC of colistin in these MBL producing organism was done using Mikrolatest Microbroth dilution test (Erba Lachema s.r.o., Karasek, Brno CZ).

60 ul of bacterial suspension in Muller Hinton broth of 0.5 McFarland standard was inoculated in each well containing serial dilution of colistin (0.25 – 16 mg/l). The inoculated strip is incubated at 37^0C for 16 – 20 hours. MIC is the lowest concentration of antibiotic in a well where no visible growth of the organism is observed. According to EUCAST interpretation table and CLSI document M100 – S28 MIC <= 2 mg/l is susceptible, and MIC >= 4 mg/l is resistant to colistin. *Ecoli* ATCC 25922 was used as control (MIC between 0.25 – 2 mg/l). For the Statistical analysis IBM SPSS version 29 has been used to check out the results.

3. Result

Among all the clinical isolates 55.87% (1170/2094) were Gram negative isolates. 26.6% (312) of these isolates were found to be resistant to Imipenem (Table 1).

Table 1: Imipenem resistance in Gram negative isolates

Isolates	Total no.	Resistant to Imipenem (%)
Ecoli	368	62 (16.85)
Klebsiella sp.	444	154 (34.68)
Pseudomonas sp.	240	52 (21.66)
Acinetobacter sp.	118	44 (37.29)
Total	1170	312 (26.66)

312 Carbapenemase producing isolates were tested for MBL production. 20.5 % of these were MBL producers (Table 2).

Table 3 shows MIC of colistin in MBL producing isolates.

Table 2: Percentage of MBL producing isolates

Isolates	Total	MBL	MBL%
Ecoli	62	8	12.90
Klebsiella sp.	154	25	16.23
Pseudomonas sp.	52	17	32.69
Acinetobacter sp.	44	17	38.64
Total	312	64(20.51%)	

Table 3: MIC colistin range in MBL producing isolates.

Isolates	MBL	MIC colistin range	Resisitant isolates	Percentage %
Ecoli(8)	8	0.25- 1	0	0
Klebsiella sp.(25)	25	0.25- 16	1	4
Pseudomonas sp.(17)	17	0.5-4	1	5.89
Acinetobacter sp.(17)	17	0.25- >=16	3	17.64
Total	64		4	6.25

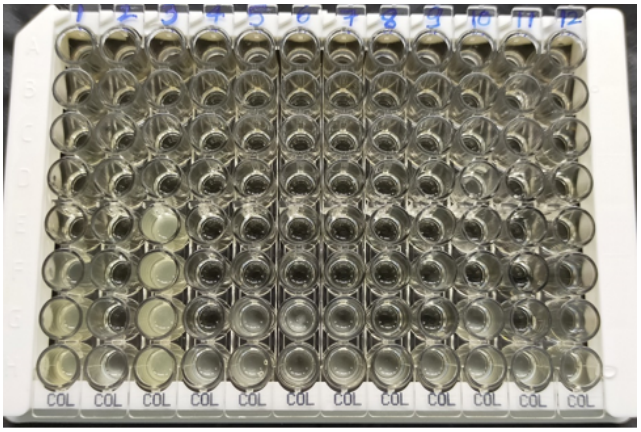

Figure 1: Shows broth microdilution (BMD) test for MIC Colistin.

4. Discussion

In our study, carbapenemase production was seen in 26.6% of total isolates (Table 1). 16.8% *Ecoli*, 34.68% *Klebsiella*, 21.6% *Pseudomonas* sp. and 37.28% *Acinetobacter* sp. were carbapenemase producers. In a recent study carbapenemase was found in 87% *Klebsiella pneumonia*.[7] (which doesn't match with present study) and only 7.9 % *Ecoli* produced carbapenemase as compared to 16.8% in present study.

12.9% of *Ecoli* were MBL producer by disc potentiation test. 16.2% *Klebsiella* sp. were found to be MBL producer. Whereas 32.6% and 38.6% were MBL producers in *Pseudomonas* sp. and *Acinetobacter* sp. respectively. A recent study reported 21.8% MBL in *Pseudomonas* sp.[8] which is lower than our study. Another study reported 20.8% MBL producing *pseudomonas* sp. which is lower than our study.[9] Others have reported 69.5% and 61.5%

which is higher as compared to present study.[10,11]

44.8% *Acinetobacter* sp. were reported to produce MBL in a recent study, similar to present one.[8] High percentage of MBL production in *Acinetobacter* sp. at 96.6% and 74% respectively has being reported.[12,13]

In a recent study, 27% of Enterobacterales and 38.6% *Pseudomonas* sp. were MBL producer[1] which is similar to ours.

Table 3 shows colistin resistance in *Acinetobacter* sp. at17.64% (MIC>=16). One isolate each of *Klebsiella* sp. and *Pseudomonas* sp. were resistant to colistin with MIC >=16 and 4 respectively. All *Ecoli* isolates were susceptible to colistin (MIC 0.25-1). Total colistin resistance found is 6.25%. 9% of Enterobacterales send to Center for Disease Prevention and Control were resistant to colistin.[14] 8.8% of *Klebsiella pneumoniae* isolates are colistin resistant according to EARS-NET report. These findings are like our study, but more elaborate study with larger sample size is needed. Colistin resistance was over 30% of CRE isolates from Italy, Spain and Greece,[15] which is higher rate of colistin resistance as compared to present study. We included only MBL producing organisms in this study, which are showing good sensitivity to colistin in present study except for *Acinetobacter* sp. which showed 17.64% resistance. We have not included class A and class D Carbapenemases in this study, which could have resulted in lower resistance to colistin in our study.

Resistance to polymyxin can be due to following reasons

1. Modifications of the LPS moiety.
2. Mutational loss of the LPS.
3. Mutations in porin and efflux pump overexpression.
4. Capsular polysaccharide (CPS) trap polymyxins and increased production of CPS in some GNB that hide the polymyxin binding sites.
5. Enzymatic inactivation of colistin.[16]

Skipped well phenomenon was seen in *Acinetobacter* sp. and *Klebsiella* sp. in the present study. Heteroresistance is observed as 'skipped wells' in colistin sensitive strains of A. baumannii, *P. aeruginosa*, *E. cloacae* and in *K. pneumoniae*. Under selective colistin pressure, resistant subpopulation develops from colistin susceptible strains. This leads to high deviation in MIC, where in MIC changes from sensitive to resistant. This is a drawback for MIC determination by BMD method. Agar dilution (AD) is also found to be reliable method for MIC determination. Agar plates stored for I week show reproducible results.[17] In another study 5 isolates resistant by BMD were shown as susceptible by Vitek 2 and AD, which is a VME (very major error).[18]

Compared to BMD, E test and disk diffusion test of colistin show erratic results. This is due to poor diffusion of colistin in the agar medium.[19,20] Colistin adhesion to variety of materials including plastic used in BMD, decreases its concentration in the well. Concentration of colistin in the experiment well depends on, 1. Material used, 2. Number of dilutions made and 3. Concentration used.[20,21] In conclusion, because of above mentioned challenges, combination of tests need to be used to determine the MIC of colistin. MBL producing organism have shown good sensitivity to colistin in present study, therefore can be used for treating such infections.

On performing running "t" test we found that the mean difference for MBL in *Ecoli*, Klebseilla sp., *Pseudomonas* sp. and *Acinetobacter* sp. is more than the MIC Colistin range. Hence we found that the isolates are more sensitive to colistin. Very few isolates were found to be resistant, which does not hold statistical importance as the p value came as <0.001. Hence we found more sensitivity of MIC colistin in MBL producing Gram negative bacilli. Hence the study shows significant results.

5. Conclusion

MBL producing gram negative bacilli show good sensitivity to colistin. But resistant subpopulation develops under selective colistin pressure which probably could affect performance of colistin in vivo.

6. Source of Funding

None.

7. Conflict of Interest

None.

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