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Re-Visiting the Last Resort: A Study of Colistin Susceptibility in Carbapenem Resistant Gram Negative Bacilli

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ABSTRACT

Increasing antibiotic resistance in multidrug resistant (MDR) gram negative bacteria contributes to increased morbidity and mortality. Colistin, a repurposed drug, has appeared as a last resort of treatment in the mid-1990s against MDR gram negative bacteria but unfortunately, rapid global resistance towards colistin has come out following its re-emergence. So it is very important to detect the colistin susceptibility in carbapenem resistant gram negative bacteria. An observational, cross-sectional study was conducted in the Bacteriology Unit, Department Of Microbiology at the Calcutta School of Tropical Medicine. Clinical samples were collected from the indoor patients for a period of 2.5 months. Isolation of carbapenem resistant gram negative bacteria along with antimicrobial susceptibility testing was performed using conventional laboratory methods as well as by automated Vitek 2 compact system. Subsequently broth microdilution was done for detection of colistin susceptibility. Results were tabulated and analyzed using WHONET software. During the study period, 461 samples showed growth in culture, of which 182(39.4%) were gram negative bacilli.73 isolates (40%) showed carbapenem resistance. Four isolates were found to be resistant to colistin by automated methods (Vitek 2). However by colistin broth micro dilution method, all isolates exhibited 100% susceptibility to colistin. The high incidence (40%) of carbapenem resistance in this study is ominous. Both false susceptible as well as false colistin resistant reports should be viewed equally gravely. Further studies are required to establish an agreeable method for detection of colistin susceptibility.

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Key Words
MDR, colistin, carbapenem and microtitre

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INTRODUCTION

Colistin has been available for clinical use since the 1960s.However, during the 1970s, this drug was largely replaced due to its high rates of nephrotoxicity[1].The recent years have seen a repurposing of this old drug in the treatment of infections caused by multidrug resistant gram negative bacilli[2]. The rapid rise of antimicrobial resistance all over the world has caused the available repertoire of treatment options to shrink to a bare minimu[3]. There are around 700,000 deaths annually that are attributable to antimicrobial resistance[4]. This figure is estimated to increase to a staggering 10 million by the year 2050[3].

Carbapenem resistant (CR) gram negative bacterial infections are of particular concern due to their high rates of mortality and morbidity. There are very few antimicrobials that have retained activity against carbapenem resistant gram negative infections such as aminoglycosides, tigecycline and ceftazidime/avibactam[4]. Polymyxins, especially polymyxin E or colistin has emerged as a last resort antibiotic for the former. Colistin is a cationic polypeptide acts by binding to the Lipid A of gram negative lipopolysaccharide resulting in loss of membrane integrity and cell death[5].

Unfortunately, with the increase in consumption of colistin, there are global reports of colistin resistant strains. Since 2015, plasmid mediated resistance by mcr-1 gene has been documented to cause colistin resistance[6]. The prevalence of colistin resistance is different in different geographical locations[7].

The challenges faced in the use and emerging antimicrobial resistance in colistin is compounded by the lack of standardized testing methods for colistin susceptibility. The presence of myriad limiting factors like multicomponent composition, poor diffusion in agar medium, synergistic effect with P-80 and last but not the least, adsorption to microtitre plates, all have variously affected the performance of various methods in its susceptibility testing[8]. In the year 2015, both the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) joint working group recommended the implementation of colistin broth microdilution method as a reference method[9]. There are other methods reported by various literatures such as agar dilution method, colistin NP Test and E test[10].

As far as automated methods are concerned, there are limited methods testing the performance of Vitek 2 in the testing of colistin susceptibility[11]. Nevertheless, the practical challenges faced while performing the broth microdilution method necessitates the evaluation of a faster, automated method. The difficulties include requirement of human resources, high cost, and lengthy time of testing[12]. This is also complicated by the fact that most laboratories, especially those in resource poor settings will only proceed to perform a BMD, when requested or as in the case of our study, after detecting carbapenem resistance [13]. The subsequent delay in reporting of the colistin MICs by broth micro dilution method makes the inclusion of this test as part of routine antibiogram panels, often impractical[14].

However, there is a worldwide trend of increase in colistin MICs, in addition to emergence of colistin resistance. This reinforces the need of a reliable and robust method for testing of colistin susceptibility [15]. There is a dearth of studies, especially from low and middle income countries regarding the formulation of a precise method for detection of colistin susceptibility. In view of this, we aimed to study the incidence of carbapenem resistant gram negative bacilli and subsequently detect colistin susceptibility in the samples.

MATERIALS AND METHODS

Study design: This was a prospective, observational, hospital-based, cross-sectional study conducted for 2.5 months(1st November,22-15th Jan,23).

Place of study: Bacteriology Unit, Department of Microbiology and Carmichael Hospital For Tropical Diseases, Calcutta School of Tropical Medicine.

Period of study: 2.5 months(1st November,22-15th Jan,23).

Collection of samples: Urine,blood,lower respiratory tract samples, pus and other relevant samples were collected by sterile, aseptic techniques from patients admitted in the inpatient department of the Carmichael Hospital and Tropical Diseases, Calcutta School of Tropical Medicine.

Exclusion criteria:

- Carbapenem sensitive gram negative bacteria
- Gram negative bacteria those are intrinsically resistant to colistin like *Serratia marcescens, Proteus sp, Morganella sp* etc

Identification of carbapenem resistant gram negative bacilli:

- Identification of gram negative isolates was performed using manual and automated methods using standard laboratory guidelines
- Testing of gram negative bacilli(Enterobacterales and non-fermenters) was carried out against the following classes of antibiotics by manual (Kirby

Bauer Disc Diffusion Method and Vitek 2) using CLSI M100 (2022) guidelines: aminoglycosides, fluoroquinolones, ampicillin, cephalosporins, tetracycline, monobactams, fosofomycin, carbapenems (meropenem, ertapenem and imipenem) and polymyxins
- An isolate exhibiting resistance to any of the 3 carbapenem drugs (carbapenem,ertapenem or imipenem) was included as carbapenem resistant
- Carbapenem resistant gram negative bacilli were preserved in 15% glycerol at-20^{0}C

Testing for colistin susceptibility by broth microdilution method:

The following steps were carried out to perform broth microdilution method for detecting colistin MICS[9]:

- Broth microdilution was performed on the carbapenem resistant isolates
- Stock solution (1000 ug mL^{-1} or 1mg mL^{-1}) of colistin sulphate salt (Sigma Aldrich, C4461) were prepared in distilled water
- Working solutions (0.03-16 ug mL^{-1}) was made by twofold serial dilutions
- U bottomed 96 well polystyrene plates were used (Tarsons)
- In each well, 100 microlitre of volume was prepared using 25 microlitre of the working solutions of colistin, 25 microlitre of the isolates and 50 microlitre of double strength cation adjusted muller hinton broth (CA-MHB)
- The plate was incubated at 37^{0}C for 16-20 hrs
- The lowest concentration of the drug the inhibited visible concentration of the antibiotic was recorded at the MIC or minimum inhibitory concentration
- **positive control:** *Escherichia coli* NCTC (13846)
- **Negative control:** ATCC *Escherichia coli* (25922)
- The results of broth microdilution test were interpreted using the EUCAST 2016 guidelines
- =< 2 ug mL^{-1} was considered sensitive
- =>4 ug mL^{-1} was considered resistant

Testing for colistin susceptibility by vitek 2[16]: The VITEK 2 system contains microlitre quantities of antibiotics and test media in plastic reagent cards. There is testing of colistin concentrations from 0.5-16 ug mL^{-1}. It is based upon the principle of turbidometry.

Data analysis: Data for isolates was entered in Who net. Thereafter analysis was performed using who net and Microsoft excel.

RESULTS

During the study period,1675 samples were received 461 cultures were positive for growth during this period of which 182 were gram negative isolates. Seventy three isolates (40%) were carbapenem resistant *Enterobacterales*,*Pseudomonas* sp and *Acinetobacter* sp. of the 73 isolates, majority were recovered from the inpatient department (67.1%) than the critical care unit (32.8%) (Table 1). Maximum number of samples, from which carbapenem resistant gram negative bacilli were recovered was urine (47.9%) followed by lower respiratory tract samples (sputum and tracheal aspirates) (32%) (Table 2).

As far as the organisms are concerned, there is a predominance of *Klebsiella pneumoniae*(45.2%) as a carbapenem resistant gram negative bacilli followed by *Acinetobacter sp* (27.3%) and *Escherichia coli* (21.9%) (Table 3). The MIC of meropenem in case of *Klebsiella pneumoniae* was more than 16 ug mL^{-1} in 100% of the isolates,followed by *Escherichia coli*,were MIC of more than 16 was observed in 94% of the isolates (Table 4) 100 % of the isolates were multidrug resistant Multidrug resistance was defined as acquired resistance to at least one agent in 3 or more antimicrobial classes.

The MIC distribution of colistin in various isolates as performed by vitek 2 and broth microdilution method has been depicted in Table 5 and Table 6. In case of *Pseudomonas aeruginosa* and *Enterobacter cloacae*, the colistin MIC of all isolates by broth microdilution method were found to be concordant with that in Vitek However in 4(5.4%) isolates *(Escherichia coli, Pantoea agglomerans, Klebsiella pneumoniae* and Acinetobacter haemolyticus) the colistin MIC as performed by VITEK was more than 16 ug mL^{-1} (resistant) while it was susceptible in case of broth microdilution method Table 7.

Essential agreement was calculated as the percentage of bacteria having MIC value within + 1 twofold dilution of broth microdilution method[17]. Categorical agreement was calculated as the percentage of isolates with results in the same category as broth microdilution method[17]. An isolate showing susceptible by reference method but resistant

Table 1: Depicting the distribution of carbapenem resistant isolates in inpatient department and critical care unit

IPD/CCU	No. Percentage
IPD	49 (67.1)
CCU	24 (32.8)

Table 2: Distribution of carbapenem resistant gram negative bacilli across various samples

Sample	No. Percentage
blood	11 (15)
catheter tip	1 (1.3)
pus	3 (4)
sputum	13 (18)
tracheal aspirate	10 (14)
urine	35 (47.9)

Table 3: Table showing different carbapanem resistant isolates

Isolate	No. Percentage
Acinetobacter sp	20 (27.3)
Pseudomonas aeruginosa	1 (1.3)
Escherichia coli	16 (21.9)
Klebsiella pneumoniae	33 (45.2)
Enterobacter cloacae	2 (2.73)
Pantoea agglomerans	1 (1.3)

Table 4: Table showing number and percentage distribution of MICs in meropenem

	MIC (ug mL^{-1})	
Isolates	>=8 (n)	>=16 (n)
Acinetobacter sp	2(10%)	18 (90%)
Pseudomonas aeruginosa	0 1 (100%)	
Escherichia coli	1 (6%)	15 (93.75%)
Klebsiella pneumoniae	0 33 (100%)	
Enterobacter cloacae	1 (50%)	1 (50%)
Pantoea agglomerans	0 1 (100%)	

Table 5 : Distribution of colistin MIC (ug ML^{-1})according to Vitek

Isolates	> = 16 (n)	8 (n)	4 (n)	2 (n)	1 (n)	< = 0.5 (n)
Acinetobacter sp	1	0	0	0	0	19
Pseudomonas aeruginosa	0	0	0	0	0	1 (100%)
Escherichia coli	1 (6%)	0	0	0	0	15 (93.75%)
Klebsiella pneumoniae	1 (3%)	0	0	0	0	32 (96.9%)
Enterobacter cloacae	0	0	0	0	0	2 (100%)
Pantoea agglomerans	1 (100%)	0	0	0	0	0

Table 6: Distribution of colistin MIC (ug ML^{-1}) according to broth microdilution method

Isolates	> = 16(n)	8 (n)	4 (n)	2 (n)	1 (n)	< = 0.5 (n)
Acinetobacter sp	0	0	0	0	1(5%)	19 (95%)
Pseudomonas aeruginosa	0	0	0	0	0	1 (100%)
Escherichia coli	0	0	0	0	0	16 (100%)
Klebsiella pneumoniae	0	0	0	1(3%)	0	32 (96.9%)
Enterobacter cloacae	0	0	0	0	0	2 (100%)
Pantoea agglomerans	0	0	0	0	0	1 (100%)

Table 7: Colistin MIC as performed by vitek was more than 16 ug mL^{-1}

Essential agreement	Categorical agreement	Major errors
69 (94.5%)	69 (94.5%)	4 (5.47%)

by Vitek was taken to be a major error. Therefore in 5.4% of the isolates, major disparity is noted in terms of MIC of colistin is detected by vitek and broth microdilution method. Four isolates showed skip wells, however on repeating test, there was no persistence of the skip wells.

DISCUSSIONS AND CONCLUSION

According to the recent reports of the ICMR-AMR Surveillance Network, resistance to imipenem was found in 28% of *Escherichia coli*, *55% of Klebsiella pneumoniae* and 80% of *Acinetobacter baumanii* isolates[4]. Thus gram negative pathogens like *Enterobacterales, Pseudomonas aeruginosa* and *Acinetobacter baumanii* are a growing cause of nosocomial infections in tertiary care hospitals[4].Carbapenem resistance exhibited by such organisms continues to limit the available treatment options while increasing the mortality and morbidity of the patients admitted. In our study, carbapenem resistance was found in 40% of the gram negative pathogens. Sadly, within years of reuse of this drug, reports of colistin resistance has also surfaced. The resistance can be attributed to both adaptive as well as plasmid mediated mechanisms[18].Plasmid mediated colistin resistance also find its culpability in indiscriminate veterinary use[19].

Our study, used broth microdilution as a reference method which is the current recommendation. Vitek 2 showed excellent essential and categorical agreement (94.5%) with broth microdilution method. This is in concordance with studies performed by Piewngam *et al*.[20] and Dafopoulou *et al*.[21] According to a study performed by Tan *et al*.[22] no false resistance was observed in Vitek 2[22]. However, our findings corroborate with the findings of Agrawal *et al*.[23] where 4% of the isolates showed susceptibility by broth microdilution method.

In the Indian context, there are hardly any reports of detection of mcr-1 gene by PCR[24].There are reports of mutation of mgrB gene.[25] Our study is limited by the absence of molecular studies in order to confirm the resistance mechanism in carbapenem resistant isolates. The microbiological profile of the isolated organisms showed a predominance of *Klebsiella pneumoniae*(45.2%),which corroborates with the findings of Pawar *et al*.[26] In concordance with Kar *et al*.[27] our study also showed a high rate of multidrug resistance(100%) in all the isolates. Our study was also limited by the absence of other testing methods for MIC of colistin and the short time line and sample size.

The present study showed that VITEK was a reliable method for detection of colistin susceptibility, however the major errors in 5.47% of the isolates warrant the use of a reference method for detection of colistin susceptibility. This is especially keeping in the often indiscriminate use of colistin in intensive care units. Antibiotic consumption should be audited and better antimicrobial stewardship practices should be implemented.

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