

***In vitro* efficacy of Colistin against multi-drug resistant *Pseudomonas aeruginosa* by minimum inhibitory concentration**

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Abstract: Multi-drug resistant bacteria are an important cause of mortality and morbidity. In the management of various infections, timely detection and appropriate treatment, in accordance with the culture and sensitivity reports can help improve the treatment outcome. Colistin is a bactericidal antibiotic which is emerging as a reliable solution for treating infections with multi-drug resistant Gram negative bacilli. The aim of this study was to find out the *in-vitro* efficacy of colistin against multidrug resistant *Pseudomonas aeruginosa* isolates by minimum inhibitory concentration. This cross sectional, descriptive study was conducted in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Islamabad from February 2010 to January 2011. Antimicrobial sensitivity testing was done on *Pseudomonas aeruginosa* isolated from routine clinical specimens received and the strains which appeared resistant to at least one antimicrobial agent in three or more anti-pseudomonal antimicrobial categories were subjected to the Colistin Etest. The MIC endpoint of colistin was read, as per manufacturer's instructions (AB Biodisk, Solna, Sweden). The isolates showing MIC of 2µg/ml or less were considered sensitive, those with 4-6µg/ml as intermediate and ≥8µg/ml as resistant. MIC₅₀ and MIC₉₀ of colistin against MDR *Pseudomonas aeruginosa* was determined. A total of 52 MDR *Pseudomonas aeruginosa* strains were isolated during the period of the study. The highest percentage was isolated from urine (36%) followed by respiratory tract infections (18%) and pus specimens (20%). The highest percentage of these isolates was found to be susceptible to colistin followed by piperacillin-tazobactam and cefoperazone-sulbactam. A total of 36(69%) isolates were sensitive, 10(20%) were intermediate and 6(11%) were resistant to colistin by Kirby Bauer disc diffusion method. MIC₅₀ was found to be 1.0µg/ml while MIC₉₀ was 3.0µg/ml. Colistin is a reliable solution in cases of infections with MDR, XDR or PDR *Pseudomonas aeruginosa*.

Keywords: Anti-biogram, multi drug resistant *Pseudomonas aeruginosa*.

INTRODUCTION

The injudicious use of broad-spectrum antibiotics has led to a rapid emergence of resistance in different pathogenic bacteria against various antibiotics (Lister *et al.*, 2009, Nadeem *et al.*, 2009, Zia-UI-haq *et al.*, 2011). *Pseudomonas aeruginosa* is one such organism which develops resistance very easily (Nadeem *et al.*, 2009). Multi-drug resistance (MDR) *Pseudomonas aeruginosa* are defined as those isolates of *Pseudomonas aeruginosa* which are found resistant to at least one antipseudomonal agent in three or more anti-pseudomonal antibiotic groups (Rossolini and Mantengoli, 2005). Various mechanisms by which *Pseudomonas aeruginosa* develops resistance are efflux pumps, biofilm formation and mutations in various chromosomal genes (Nadeem *et al.*, 2009, Tam *et al.*, 2010). Multi-drug resistance (MDR) in bacteria leaves us with limited and expensive treatment options (Heijden *et al.*, 2007). The incidence of MDR *Pseudomonas aeruginosa* is reported to be 5.8% by Cholley *et al.* (Cholley *et al.*, 2010).

The bactericidal antibiotic colistin, is a cationic polypeptide which acts by disrupting membrane functions of active transport (Brookes *et al.*, 2007, Kaye and Kaye,

2004). It was not being commonly used because of its neurotoxic and nephrotoxic effects but now it is observed to have a good efficacy against MDR *Pseudomonas aeruginosa* strains (Brookes *et al.*, 2007). Proper dose adjustments may help prevent the renal side effects of this useful antimicrobial agent (Clinical and Laboratory Standard Institute, 2010).

In the management of various infections, timely detection and appropriate treatment, in accordance with the culture and sensitivity reports can decrease the mortality, morbidity and the expenditure of the patient (Nadeem *et al.*, 2009). Estimation of minimum inhibitory concentration (MIC) by broth micro-dilution method has been recommended for detecting the *in vitro* susceptibility of colistin by Clinical and laboratory standards institute (CLSI) (Clinical and Laboratory Standard Institute 2010). Etest is a standardized method of MIC determination having 100% coherence with broth micro-dilution method (Brookes *et al.*, 2007). The knowledge of susceptibility pattern of colistin against MDR *Pseudomonas aeruginosa* is very essential for the clinicians for proper management of their patients. The current study was planned to find out the *in vitro* efficacy of colistin against multidrug resistant *Pseudomonas aeruginosa* isolates by minimum inhibitory concentration.

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MATERIALS AND METHODS

This cross sectional, descriptive study was conducted in the Department of Microbiology, Army Medical College, Rawalpindi from January 2010 to December 2010. Routine clinical specimens including blood, urine, pus, sputum and nasobronchial lavage (NBL) received in the Department of Microbiology, Army Medical College, Rawalpindi, Pakistan, were applied on appropriate culture media like blood, chocolate or MacConkey agars (Oxoid, U.K.) and the pathogens were identified to species level by standard microbiological methods like colony morphology, Gram stain, pigment production, oxidase test and analytical profile index (API-20NE), if required. Antibiotic susceptibility pattern was determined by using Kirby Bauer disc diffusion method using antibiotic discs of meropenem (10µg), Imipenem (10µg), Amikacin (30µg), Gentamicin (10µg), Piperacillin-tazobactam (110µg), Cefoperazone-sulbactam (105µg), Aztreonam (30µg), Colistin (10µg), Polymixin-B (300units), Cefoperazone (75µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Tobramycin (10µg) were applied on the inoculated MHA(Mueller Hinton Agar) plates. The plates were then incubated at 37°C for 24 hrs. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standard Institute 2010).

The isolates which appear resistant to at least one antimicrobial agent in three or more anti-pseudomonal antimicrobial categories, so defined as MDR *Pseudomonas aeruginosa*, were subjected to the Colistin Etest.

Colistin Etest

MIC of colistin was determined by Colistin Etest. A 0.5 McFarland suspension of the MDR *Pseudomonas aeruginosa* isolate was prepared and applied on the MHA plate. Colistin Etest strips (AB Biodisk, Solna, Sweden) was applied on the plate and it was then incubated at 35±2°C for 16-18hrs. The MIC endpoint of Colistin was read at the tapering end of the ellipse, as per manufacturer's instructions (AB Biodisk, Solna, Sweden) (Customer Information Sheet Etest and Polymyxin B testing, Etest Antimicrobial susceptibility testing for in vitro antibiotic diagnostic use). The isolates showing MIC of 2 µg/ml or less were considered sensitive, those with 4-6 µg/ml as intermediate and ≥8µg/ml as resistant (Clinical and Laboratory Standard Institute 2010).

Table 1: Percentages (%ages) of MDR *Pseudomonas aeruginosa* isolates with different Minimum Inhibitory Concentration (MIC) values alongwith MIC₅₀ and MIC₉₀

MIC (µg/ml)	0.125	0.19	0.38	0.50	0.75	1.0	1.5	2.0	3.0	4.0	MIC Range (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
% age	2.5	5.0	2.5	7.5	7.5	30.0	7.5	12.5	17.5	7.5	0.125-4.0	1.0	3.0

RESULTS

A total of 46 MDR *Pseudomonas aeruginosa* strains were isolated during the period of the study. The highest percentage of these strains was isolated from urine followed by respiratory tract infections. Regarding the antimicrobial susceptibility, the highest percentage of these isolates was found to be susceptible to colistin (67%) followed by piperacillin-tazobactam (64%) and cefoperazone-sulbactam (37%). The isolates were least susceptible to imipenem, gentamicin and quinolones. A total of 31 (67%) isolates were sensitive, 10 (22%) were intermediate and 8 (17%) were resistant to colistin (fig. 1). MIC determined by Etest (fig. 2) had a range of 0.125-4.0µg/ml (table 1). MIC₅₀ (Concentration of antibiotic required to inhibit the visible growth of 50% of the organisms) was found to be 1.0 µg/ml while MIC₉₀ (Concentration of the antibiotic required to inhibit the visible growth of 90% of the organisms) was 3.0 µg/ml (table 1).

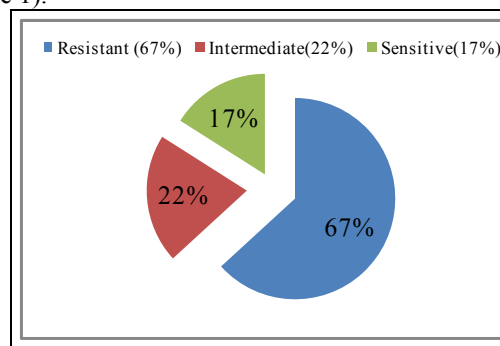


Fig. 1: Percentage Susceptibility of the MDR *Pseudomonas aeruginosa* strains against Colistin



Fig. 2: MIC value of an isolate of MDR *Pseudomonas aeruginosa* determined by Etest.

DISCUSSION

The emergence of MDR *Pseudomonas aeruginosa* is a matter of great concern for the clinicians worldwide. There is no standard definition of MDR *Pseudomonas aeruginosa* till now (Rossolini and Mantengoli, 2005). Obritsch *et al* and Hachem *et al* have defined MDR *Pseudomonas aeruginosa* as the isolates resistant to three of the agents like β -lactam (piperacillin, piperacillin-tazobactam, cefipime, ceftazidime, ticarcillin, ticarcillin-clavulanic acid), carbapenems (imipenem, meropenem), aminoglycosides (gentamicin, tobramycin, amikacin) and flouroquinolone (ciprofloxacin) (Obritsch *et al.*, 2005, Hachem *et al.*, 2007). Falages *et al*, on the other hand have defined all those isolates of *Pseudomonas aeruginosa* as MDR which were found resistant to at least five out of seven anti-pseudomonal categories like penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides and colistin (Falages *et al.*, 2005). We have taken the definition from two online documents; one was prepared, by a group of experts, from European Center for Disease Prevention and Control (ECDC) and Centre for Disease Control and Prevention (CDC) and the other was a reply on this document from the American Society of Microbiology (Magiorakos *et al.*, 2010, ASM 2010).

For the management of infections with MDR, XDR (extremely-drug resistant) or PDR (Pan-drug resistant) *Pseudomonas aeruginosa* where the treatment options are quite limited the older drugs like colistin are emerging as a reliable solution. Colistin or Polymyxin-E is found to have dose-related neurotoxic and nephrotoxic effects (Kaye and Kaye 2004). The dose adjustment in patients with renal impairment can help minimize these side effects (Kaye and Kaye 2004). The dose for patients with normal renal function is 80-160mg/8hrs (Gilbert *et al.*, 2006). The dose of colistin in patients with estimated creatinine clearance (CrCl in ml/min) of >50-90, 10-50 and <10 should be reduced to 160 mg/12hrs, 160mg/24hrs and 160mg/36hrs respectively (Gilbert *et al.*, 2006).

Taking a look on the studies conducted in various parts of the world, we find, Obritsch *et al*, in a review article are of the view that with monitoring of the toxic effects, colistin can be used in combination with rifampicin or β -lactam drugs for the treatment of infections with MDR *Pseudomonas aeruginosa* (Obritsch *et al.*, 2005). In our study, we have found MIC₅₀ of 1 μ g/ml and MIC₉₀ of 3 μ g/ml. Our results match with a study conducted in Riyadh (2010), where Somily have found MIC₉₀ of colistin against MDR *Pseudomonas aeruginosa* to be 3 μ g/ml (Somily, 2010).

Thus for the infections with MDR strains of *Pseudomonas aeruginosa*, can be life-saving, in proper dosage and proper route of administration. We recommend more

large-scale in-vivo studies for the evaluation of the efficacy of colistin against MDR *Pseudomonas aeruginosa*.

CONCLUSION

Colistin is a reliable option for the treatment of infections caused by MDR isolates of *Pseudomonas aeruginosa* with proper dose adjustment.

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