

In vitro Efficacy of Colistin by E. Strip and Agar Dilution Method against Multi-drug Resistant Gram negative Rods Isolated from Clinical Specimens in Tertiary Care Hospitals

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ABSTRACT

Background: Resistance among bacterial isolates is one of the major reasons of increased mortality and morbidity worldwide. MDR Gram-negative pathogens are more common in exhibiting resistance against available antimicrobials. Older antimicrobial are increasingly in use now a days due to lack of new and effective antibiotics. Colistin is an older antimicrobial which has significant post antibiotic effect against MDR Gram-negative pathogens. The knowledge of susceptibility pattern of Colistin against MDR-GNB is very essential for the clinicians. The rapid and reliable methods for Colistin susceptibility testing are necessary due to increase in its use worldwide. The purpose of this study was to observe the Colistin susceptibility of MDR-GNB isolates in our setup by simple and reliable method.

Methods: The study analyzed eighty eight (88) MDR Gram-negative strains isolated from clinical specimens collected from two different institutions i.e., Armed Force Institute of Pathology (AFIP) Rawalpindi and Department of Surgery Lahore General Hospital. The samples were tested according to the Clinical Laboratory Standards Institute (CLSI) protocol by E-strip method followed by Agar dilution method for confirmation.

Results: Out of 88 clinical specimens, 95% specimens found sensitive to Colistin showing inhibition at the susceptible breakpoints. It appears that *E. coli*, KP, *E. cloacae* and *Salmonella typhi* were 100% susceptible to Colistin by both methods, while *P.aeruginosa* and *A. baumannii* showed 90.9% and 88.8% susceptibilities respectively.

Conclusion: The results of our study showed that E-strip method is a simple, reliable and attractive alternative to reference method (Agar Dilution) for determining Colistin susceptibility and resistance in gram negative bacilli. Isolated strains of MDR gram negative bacilli are highly sensitive to Colistin in our set up.

Keywords: Colistin, E-strip, agar dilution method, multi-drug resistance.

INTRODUCTION

The worldwide rise in antimicrobial resistance of microorganisms can have devastating effect on medical research and health-care community. Multi-drug resistant gram-negative bacilli are the most common pathogens which can cause various infections in human beings. The injudicious and inappropriate use of antimicrobial therapy has led to increase resistance among pathogens.¹ Medical practitioners are left with very few options to treat MDR-GNB infected patients. Older antibiotics are being reconsidered to treat MDR-GNB infections. Among these older antimicrobial groups are Polymyxins, which were not in use since early 70s. Polymyxins now hold an important role in the antibiotic armamentarium. Considering MDR-GNB

infections O Fallon E et al³ reported prevalence of 21.8% among the healthcare workers. A tertiary care hospital from India showed 22% MDR *P.aeruginosa* from different clinical specimens⁵. An *Acinetobacter baumannii* outbreak from an intensive care unit of a tertiary care hospital Rawalpindi (Pakistan) was reported in 2011⁶. Polymyxin group comprises of polymyxins A, B, C, D, and E. Clinically polymyxin B and polymyxin E (colistin) are in use. Colistin was discovered in 1947 from *Bacillus colistinus*.⁷ Just after its introduction in 1949, toxicities associated with clinical use reported. Later it was observed that adverse effects of Colistin were reversible. Mode of action of Colistin is to bind with and LPS and phospholipids of bacterial outer cell membrane causing disruption and leakage of cell contents leading to cell death⁸. Colistin therapy success rates were very encouraging. A study from India observed 88% cure rate in critically ill MDR-GNB infected patients¹. Another study reported Colistin cure rate of

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83.9% in pediatric patients from Turkey⁹. The increase in Colistin use over the last few years requires an accurate and reliable susceptibility testing methods.¹⁰. Antimicrobial susceptibility testing can be done by Diffusion as well as dilution techniques. The purpose of our study was to determine *in vitro* efficacy of Colistin (polymyxin E) against multi-drug resistant gram-negative bacilli isolated from clinical specimen in our setup by E.strip and Agar Dilution Methods.

MATERIALS AND METHODS

This descriptive, cross-sectional study was conducted at the Department of Microbiology, AFIP, Rawalpindi, from January 2013 to December 2013. Specimens were collected from two different institutions, CMH Rawalpindi and Department of Surgery Lahore General Hospital. Total 88 isolates of MDR-GNB (resistant to ceftriaxone, ciprofloxacin, Imipanam and Amikacin) were selected for the study by taking 80% power of study and 95% confidence level. Patients with non MDR-GNB isolates and duplicate samples were excluded. **E-Test:** Isolated colonies MDR-GNB were picked and tested by E.strip method to get an ellipse shaped zone of no growth. Enterobacteriaceae, *pseudomonas aeruginosa* and Acietobacter spp showing MIC,s equal to or less than $\leq 2\mu\text{g/ml}$, $\leq 4\mu\text{g/ml}$ and $\leq 2\mu\text{g/ml}$ respectively were considered as sensitive.

Agar Dilution Method: MICs of colistin against MDR-GNB was confirmed using agar dilution technique (as recommended by CLSI). Data was analyzed by SPSS (version 17.0) statistical package. Percentages for MDR-GNB isolates were calculated. Frequencies and percentages for types of specimens and types of bacterial isolates were calculated. Descriptive statistics were applied for both qualitative and quantitative variables. For quantitative variables like age, mean and $\pm\text{SD}$ were calculated. For qualitative variables like colistin susceptibility, frequencies and percentages were calculated.

RESULTS

Out of 88 clinical specimens 40 were from urine, 20 from pus, 12 from blood, 10 from BAL (bronchoalveolar lavage) and 6 were taken from sputum. Highest number of test strains was isolated from urine i.e., 46%. While only 7% were isolated from sputum (Fig. I). Percentages of different multi drug resistant gram-negative bacteria included in the study are shown in Figure II. All organisms were identified using Gram staining, culture and biochemical profile index. Figure IV is showing that 95% MDR-GNB susceptible to Colistin while only 5% showed Colistin resistance. Table

is presenting minimum inhibitory concentration of Colistin against different MDR-GNBs included in this study by E.strip and Agar dilution method (standard method). Figure III is presenting the percentage susceptibilities of different MDR-GNB to Colistin. It appears that *E.coli*, KP, *E. cloacae* and *Salmonella typhi* were 100% susceptible to Colistin by both methods, while *P. aeruginosa* and *A.baumannii* showed 90.9% and 88.8% susceptibilities respectively

Fig. I: Data showing percentages of different MDR-GNB samples

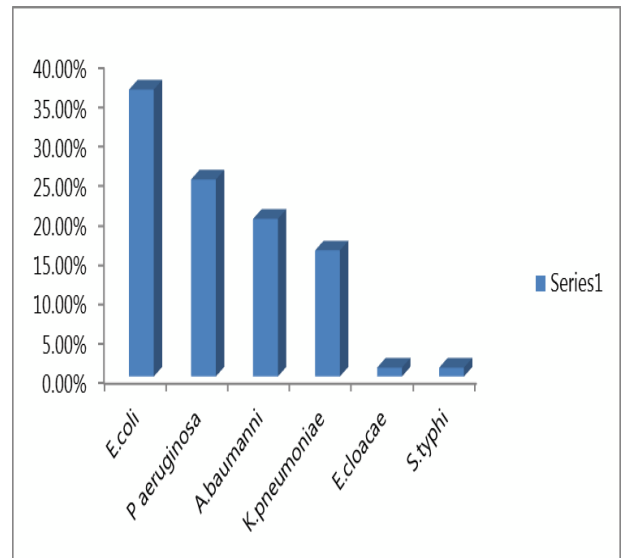


Fig. II: Percentages of different MDR-GNB

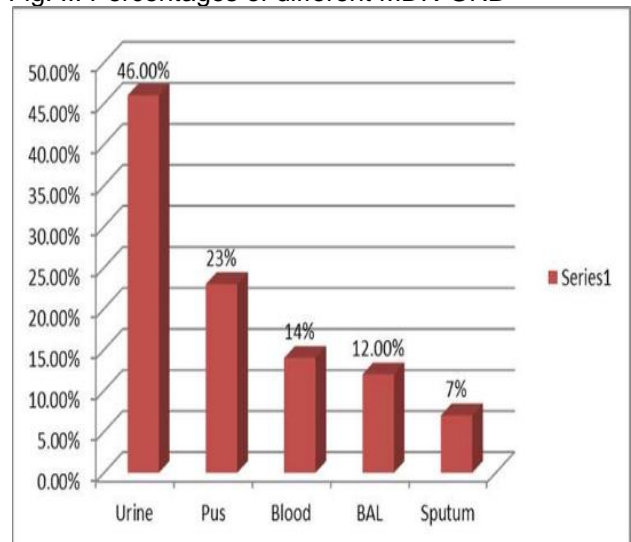


Fig. III: Percentage susceptibility of different MDRGNB

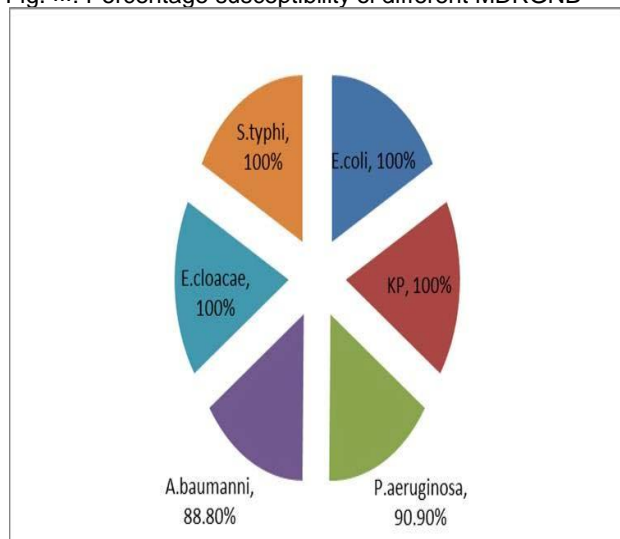


Fig IV: Colistin susceptibility

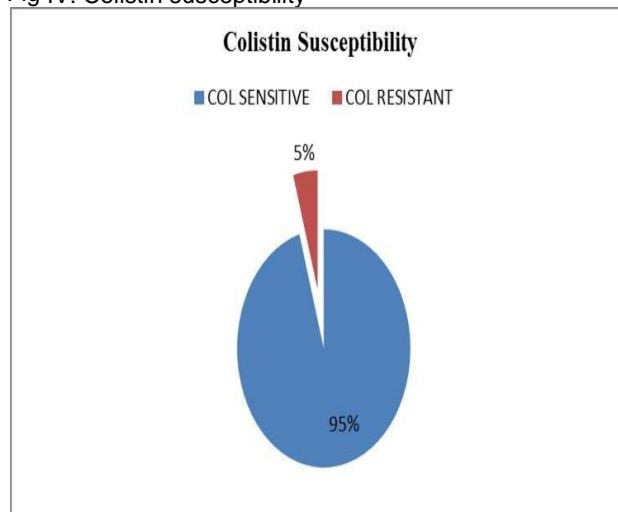


Table: Activity of Colistin against MDR-GNB shown as MIC and susceptibility by E. test and Agar dilution

Organisms n:88	No. of Organisms	MICµg/ml	Susceptibili By E. test	Resistant	Susceptibility by Agar Dilution	Resistant
E.coli	32	0.5	32	None	32	None
K.pnenoniae	14	1.0	14	None	14	None
P.eruginosa	22	1.0	20	2	20	2
A.baumannii	18	1.0	16	2	16	2
E.cloacae	1	0.25	1	None	1	None
S.typhi	1	0.25	1	None	1	None

DISCUSSION

The requirement of an effective antimicrobial agent against MDR GNB infections has led the clinicians to use Colistin (older antibiotic) worldwide. Continuous monitoring of kidney function test and serum drug levels can reduce the adverse effects of the Colistin therapy¹¹. commonly used antimicrobial such as Carbapenes can be used to confirm the Colistin effectiveness against MDR-GNB. Recently published data has shown that Colistin can perform bactericidal activity against *Acinetobacter spp*, *P.aeruginosa* and most members of the Enterobacteriaceae.¹² Our study has also confirmed these results. The purpose of our study was to generate data regarding in vitro Colistin efficacy and to assess any developing resistance against this drug in Pakistan. There are different methods to interpret Colistin susceptibilities but data available regarding accurate method is very limited. Previous studies have shown that due to some inherent properties, Colistin diffuses poorly in agar resulting in small zones of inhibition.¹³ In view of the inaccuracy of disk diffusion MIC based dilution methods might present an attractive alternative.

Using BSAC (British Society for Antimicrobial Chemotherapy) break points, Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter spp* showing MICs equal to or less than $\leq 2\mu\text{g/ml}$, $\leq 4\mu\text{g/ml}$ and $\leq 2\mu\text{g/ml}$ respectively were considered as sensitive¹⁴.

A similar study was conducted in Singapore reported that *Acinetobacter spp*, and *E. coli* were 100% to Colistin while, *P. aeruginosa* and *Enterobacter K. pneumoniae* showed 67% and 75%, 94%, susceptibilities respectively¹⁵. In our study *E. coli*, *K. pneumoniae*, *Enterobacter spp* and *S.typhi* showed 100% susceptibility to Colistin while *Acinetobacter spp* and *P. aeruginosa* showed 80.8% and 90.9% respectively. The CANWARD Study from Canadian hospitals(2007-2008) reported that more than 90% of MDR-GNBs were susceptible to Colistin¹⁷ The SENTRY Antimicrobial Surveillance Program (2006-2009) found excellent susceptibility of MDR-GNB to Polymyxins (Colistin, Polymyxin B) and also noticed low rates of resistance. They also found increase tendency of developing resistance due to mismanagement of this precious drug. They

suggested polymyxins should be used cautiously after performing susceptibility testing to avoid the resistance.¹² Colistin resistance of 3.5% among 224 urinary isolates of patients with complicated urinary tract infection was reported from a prospective study of north India.¹⁷ We noticed 5% Colistin resistance in our study. Like all other antimicrobials Colistin also require antimicrobial stewardship programs (ASPs) to prevent or slow the emergence of colistin resistance. In addition, an effective infection control program must be developed at the local level in each hospital. The effectiveness of Colistin against MDR-GNBs has been proved by different studies worldwide. Different studies have showed that toxicities of this drug can be reduced by modern patient care and proper drug monitoring. MIC of Colistin against 172 isolates of gram-negative bacilli collected from different clinical specimens was determined and compared by different susceptibility methods by T. Y. Tan and S. Y. Ng in 2007. Categorical agreement was found to be of 87% between agar dilution and E test methods.¹⁵ In our study both methods showed 100% similar results which support the idea of using E. strip method routinely in clinical laboratories to assess Colistin susceptibility. We conducted this study to find in vitro efficacy of Colistin by MIC based susceptibility testing methods. We also evaluated the performance of E. test and agar dilution test as disc diffusion was considered unreliable test by different studies. The results were very encouraging. Strict infection-control measures can minimize the development and progression of resistance against this precious drug. Any concerns and obstacles regarding infection control must be identified and properly addressed because any emerging resistant infection can breed another emerging multi-drug resistant infection. In our opinion in vitro effectiveness of Colistin needs to be ensured by careful and ongoing monitoring.

There is a need to conduct surveillance programs in every country periodically to detect any escalating tendencies of MICs. Thus, this study is helpful in establishing the role of Colistin as excellent therapeutic option against MDR-GNB infections.

CONCLUSION

It is concluded that the isolated strains of multi-drug resistant gram negative bacilli in our set up are highly sensitive to Colistin. E. test is simple, reliable and attractive alternative to reference method (Agar Dilution Method) for determining Colistin susceptibility and resistance in gram negative bacilli. In case of

critically ill patients having MIC near breakpoints, results can be confirmed by Agar dilution method. Colistin can hopefully serve as a bridge until more effective therapeutic agents are developed.

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