IN VITRO EFFICACY OF CICLOPIROX AGAINST CARBAPENEM RESISTANT ENTEROBACTERIACEAE

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ABSTRACT

Objective: To determine the in vitro efficacy of Ciclopirox against Carbapenem Resistant Enterobacteriaceae by determining its minimum inhibitory concentration by micro agar dilution method.

Study Design: Quasi experimental study.

Place and Duration of Study: The department of Microbiology, Army Medical College, Rawalpindi, National University of Sciences and Technology, Islamabad Pakistan, from Apr 2015 to Apr 2016.

Methodology: Sample size 45 Carbapenem Resistant Enterobacteriaceae. Clinical specimens like naso-bronchial lavage (NBL), blood, pus, sputum, urine, body fluids and catheter tips, routinely received in the department of Microbiology, Army Medical College, Rawalpindi were subjected to standard microbiological methods. Enterobacteriaceae were identified by colony morphology, and API 20 E (Biomeriux. France). Carbapenem resistance was detected by using imipenem/meropenem discs (10 ug) by modified Kirby bauer disc diffusion method. Ciclopirox minimum inhibitory concentration (MIC) against the selec-ted samples of Carbapenem resistant Enterobacteriaceae was measured using micro agar dilution protocol done in accordance with clinical laboratory standards institute (CLSI).

Results: All 45 (100%) carbapenem resistant enterobacteriaceae (CRE) were sensitive to ciclopirox. Forty-three out of 45 (95.5%) of carbapenem resistant Enterobacteriaceae were multi drug resistant (MDR) i.e., sensitive only to minocycline, tigecycline, and colistin. Minimum inhibitory concentration of ciclopirox was determined against multi drug resistant Carbapenem resistant Enterobacteriaceae was found to be 25µg/ml. Two (4.44%) were pan drug resistant and the Minimum inhibitory concentration of ciclopirox for these organisms was 50 µg/ml.

Conclusion: Ciclopirox is highly effective in vitro against Carbapenem resistant Enterobacteriaceae at Minimum inhibitory concentration between 25-50 µg/ml.

Keywords: Carbapenem resistant enterobacteriaceae, Enterobacteriaceae, Klebsiella pneumonia.

INTRODUCTION

The escalating rates of resistance amongst commonly occurring bacterial pathogens have emerged as a crucial challenge to human health during the previous few decades[1]. Pathogens with carbapenem resistance often demonstrate high level of resistance to other groups of antibiotics as well, such as quinolones, aminoglycosides and cephalosporins. As a result, the clinicians haveto resort to the very few choices which include tigecycline, polymyxin, Fosfomycin, and temocillin[2]. The major concern with the administration of aminoglycosides and polymyxins is nephrotoxicity. The reason for limiting the use of tigecycline is the frequent reports of increased resistance and decreased blood levels among patients. Thus, leaving no option to effectively treat the deadly infections caused by such resistant strains[3]. Therefore, development of new antibiotics against resistant strains is the need of the hour.

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Ciclopirox is an off patent antifungal drug related to Hydroxypyridone group. It has been in use as an anti-fungal since decades and not a single case of resistance against this drug is reported[4]. Ciclopirox has a very good safety profile. These favorable qualities have led to ciclopirox currently being investigated as a treatment of multiple types of malignancies[5,6], wounds in diabetes mellitus[7,8], congenital erythropoietic porphyria[9], and various viral diseases including Herpes[10], and Human immunodeficiency virus infections. Studies have shown that ciclopirox also has an antibacterial effect at different minimum inhibitory concentrations. Ciclopirox acts by inhibiting the two major pathways that include galactose metabolism and LPS biosynthesis. These pathways have vital role for bacterial growth and virulence. Its significant antibacterial activity against multidrug resistant Acinetobacter, Escherichia coli and Klebsiella pneumoniae has already been proven by a study done by Carlson-Banning. These bacteria were sensitive to carbapenems. Here, the aim of our study is to assess the efficacy of ciclopirox, against Carbapenem resistant Enterobacteriaceae (CRE)by measu-

ring its Minimum inhibitory concentration by Micro agar dilution method. Repurposing the drug that is already in use since decades will be beneficial as it circumvents time and money as compared to the cost of developing a new drug from scratch.

METHODOLOGY

This quasi experimental study was carried out at the department of Microbiology, Army Medical College, Rawalpindi, from April 2015 to April 2016. The sample size was calculated using confidence level of 95%, alpha error of 10% as used for baseline studies, study power of 80%, anticipated population proportion with resistant findings 86.5-93.4%. The study sample was 45 cases. Clinical specimens like naso-bronchial lavage (NBL), blood, pus, sputum, urine, fluid and catheter tips, routinely received in the Department of Microbiology, Army Medical College, Rawalpindi were subjected to standard microbiological methods for identification (table-III). Enterobacteriaceae were identified by colony morphology, catalase test, oxidase test and API 20 E (Biomeriux, France). Carbapenem resistance was detected by using Modified Kirby Bauer disc diffusion method by applying meropenem disc (10µg). Antibiotic sensitivity was done using modified Kirby Bauer disc diffusion technique. Minimum inhibitory concentration of ciclopirox was determined against the selected samples of Carbapenem resistant Enterobacteriaceae by using micro agar dilution protocols done in accordance with Clinical Laboratory Standards Institute (CLSI). Antibiotic sensitivity of CRE was also assessed against all the first line drugs along with colistin and tigecycline (table-II). The data entry and statistical analysis was done in SPSS-22. The continuous numerical variables were measured as mean and standard deviations whereas the categorical variables were measured as frequency and percentages.

Preparation of the standard stock solution 100mg of the ciclopirox base powder was weighed accurately on the analytical balance and transferred carefully to a 100ml volumetric flask. The pure drug in the volumetric flask was then dissolved in DMSO up to the 100ml mark to obtain a solution of concentration of 1000 µg/ml. (Stock solution). Stock solution was stored in small test tubes at-20C. Serial dilutions are made by using formula.

$C1\ V1 = C2\ V$

Serial two-fold dilution from the stock solution is shown in table-I. Two-Fold Serial Dilutions of ciclopirox.

The Meullar Hinton agar was prepared according to manufacturer's instructions. The molten agar was then mixed gently with different concentrations of antimicrobials. A drug-free control was also included. Inoculum for all 45 strains was prepared according to McFarland turbidity standards and inoculated on prepared agar and incubated at 37°C overnight.

RESULTS

Out of 1100 enterobacteriaceae 45 were CRE. Most of these were isolated from the specimen received from medical intensive care unit. All isolates included in the study were 100% resistant to carbapenems. Forty-three out of 45 (95.5 percent) were only sensitive to colistin, minocycline, and tigecycline (MDR: multidrug resistant). Two (4.4 percent) isolated from urine were resistant to all first line drugs and colistin (PDR: Pandrug resistant). Anti-biogram of Carbapenem Resistant Enterobacteriaceae to various drugs is shown in table-II.

Table-I: Two-fold serial dilutions of ciclopirox.

Concentration of Stock Solution (µg /ml)	Volume of Standard Stock Solution (ml)	Final Concentration (µg/ml)	Volume of Media Required for Each Concentration (ml)
1000	2	100	20

Table-II: Anti biogram of carbapenem resistant enterobacteriaceae to various drugs.

Antibiotics	Resistant n= 45 (%)	Sensitive n (%)
Ceftriaxone	45 (100)	-
Ceftazidime	45 (100)	-
Cotrimoxazole	45 (100)	-
Meropenem	45 (100)	-
Colistin	2 (4.44)	43 (95.5)
Minocyclines	2 (4.44)	43 (95.5)
Tigecyclines	2 (4.44)	43 (95.5)
Ciprofloxacin	45 (100)	-
Amikacin	45 (100)	-
Gentamicin	45 (100)	-
Tazocin	45 (100)	-
Sulzone	43 (100)	-

Ciclopirox showed excellent result against Carbapenem resistant Enterobacteriaceae. All 45 (100%) isolates were sensitive to ciclopirox, irrespective of the susceptibility pattern of isolates. Forty-three out of 45 were multidrug resistant and their Minimum inhibitory concentration was found to be 25µg/ml (fig-2). Minimum inhibitory concentration of Ciclopirox against Pan-drug resistant (n=2) was found to be 50µg /ml. At 12.5 µg/ml all strains were resistant and visible growth was recorded (fig-1). The mean MIC value

in this study was 26.11 ± 5.16 over a range of 6.25-100 µg/ml (table-IV).

Table-III Specimen wise distribution of carbapenem resistant enterobacteriaceae.

Source	Carbapenem Resistant Enterobacteriaceae		
	Klebsiella Pneumoniae	Escherichia Coli	Others
Blood	7	1	-
Respiratory isolates*	10	1	-
Urine	4	4	-
Pus	3	-	1 (Citrobacter fruendi)
Ascitic Fluid	1	-	-
Tip	10	-	1 (Enterobacter aerogenes)
Tissue	1	-	-
Stool	1	-	-
Total	37 (82%)	6 (13.3%)	2

Table-IV: Minimum Inhibitory concentration of ciclopirox for carbapenem resistant enterobacteriaceae.

Minimum Inhibitory Concentration (µg/ml) of ciclopirox					
	6.5	12.25	25	50	100
No. of isolates (CRE)	-	-	43	45	

DISCUSSION

The current upsurge in prevalence of Carbapenem Resistant Enterobacteriaceae (CRE) has raised concerns throughout the world. It has led to increase in health management costs not only due to increased morbidity and mortality, but also because of readmissions of those patients who survived Carbapenem Resistant Enterobacteriaceae infections[15].

In our study, out of 1100 isolates of Enterobacteriaceae, isolated during the study period, 4% were Carbapenem resistant. Most of the Carbapenem Resistant Enterobacteriaceae were Klebsiella pneumoniae 37/45 (82%) followed by *Escherichia coli* 6 (13.33%). These isolates were mainly from the severely ill patients admitted in Intensive care units and were on ventilator. Similar rise in the occurrence of Carbapenem resistant Enterobacteriaceae in China is also reported. The Carbapenem resistance of *E. Coli* and *K. pneumoniaein* 2004 to 2005 is 0-0.7%, however, in 2010, the rate escalated to 0.5% and 2.7%[16]. In another study done in Egypt in 2017, the prevalence of Carbapenem resistant Enterobacteriaceae was 54.1%[17].

In our study ciclopirox was found highly effective against carbapenem resistant Enterobacteriaceae. This is the first study that is done to know the efficacy of ciclopirox against carbapenem resistant Enterobacteriaceae. In a study done by Carlson-Banning, the efficacy of ciclopirox was studied against carbapenem sensitive bacteria including *Acinetobacter baumannii,*

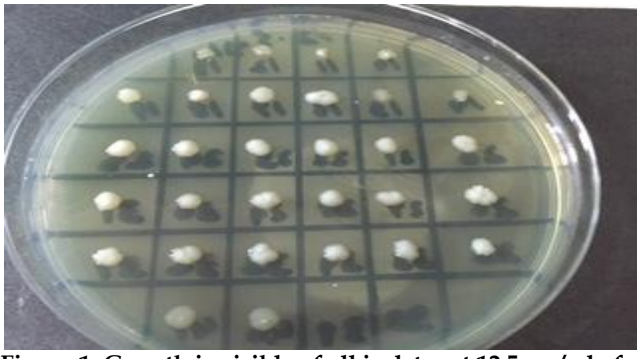

Figure-1: Growth is visible of all isolates at 12.5 µg/ml of ciclopirox.

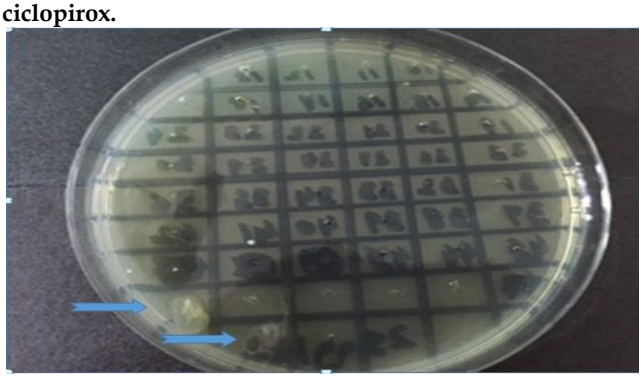

Figure-2: No visible growth at 25 µg/ml except two strains Arrows indicate growth of two PDR strains.

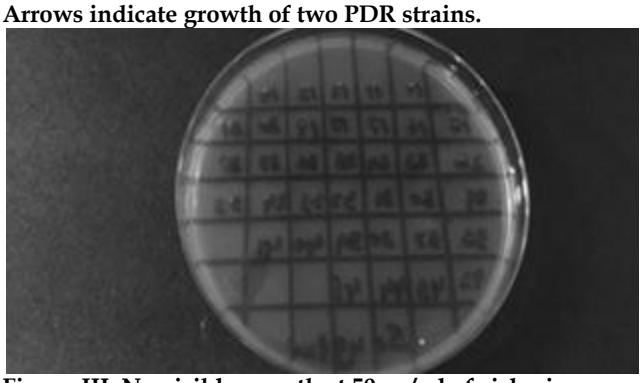

Figure-III: No visible growth at 50 µg/ml of ciclopirox.

Klebsiella pneumoniae and *Escherichia coli*. (MIC 5-15 µg/ml)[13]. Our study is a step ahead, as the significance of ciclopirox is elaborated by determining its efficacy against Carbapenem Resistant Enterobacteriaceae. The results were remarkable, as ciclopirox was found to be effective against such resistant strains. The minimum inhibitory concentration was found to be ranging from 18-25 µg/ml irrespective of the type of resistance against Carbapenems. Two of the carbapenem resistant isolates were found to be resistantto all drugs including tigecycline and colistin. Ciclopirox was effective

in vitro against these resistant strains as well and minimum inhibitory concentration for these strains was found to be 50 µg/ml. In a study done by Kim *et al*, the minimum inhibitory concentration of ciclopirox against drug sensitive Escherichia coli strains ATCC25922 and BW25113 was 25 µg/ml[18]. Another study by Shin *et al*, supports ourresults in which the antibacterial activity of ciclopirox against multidrug resistant Escherichia coli was analyzed by wide genome expressing profiling using the same range of minimum inhibitory concentration of ciclopirox (15-25 µg/ml). Conley *et al*, worked on different strains of Escherichia coli to find out the two pathways involved in the mechanism of action of ciclopirox. Most of the strains with different susceptibility pattern were sensitive to ciclopirox at minimum inhibitory concentration range of 11-25 µg/ml[19,20].

Ciclopirox has an excellent safety profile and it has successfully cleared clinical phase 1 trial for systemic administration. It is very well tolerated at the dose of 40 mg/m^2 once daily and shows no side effects. It is excreted in urine as a glucuronide metabolite[5].

Ciclopirox has been in use as an anti-fungal since decades. The emergence of ciclopirox as an antibacterial is the major breakthrough in the field of medicine as it will not only enhance the life expectancy and wellbeing of the patients but also circumvents the cost of developing a new antibiotic from scratch. Discovery of novel properties of ciclopirox hasrevived its significance in health care systemand has kindled a ray of hope for better health conditions among high risk patients.

RECOMMENDATIONS

Further trials of ciclopirox should be carried out to evaluate its efficacy in other parts of the world because it is the need of the hour to have effective treatment options against leading pathogens like Carbapenem resistant Enterobacteriaceae in this era of emerging resistance against antimicrobials.

CONCLUSION

Ciclopirox is highly effective in vitro against Carbapenem resistant Enterobacteriaceae at minimum inhibitory concentration of between 25-50µg/ml. In years to come ciclopirox will serve as an excellent option against the deadly infections caused by resistant organisms.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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