ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF POLYMYXIN B, TIGECYCLINE AND FOSFOMYCIN AGAINST CARBAPENAMASE PRODUCING ENTEROBACTERIACEAE (CPE)

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ABSTRACT

Objective: To determine, the susceptibility pattern of carbapenamase producing enterobacteriaceae (CPE) against polymyxinB, tigecycline and fosfomycin.
Study Design: Descriptive cross sectional.
Place and Duration of Study: The study was carried out in the Department of Microbiology PNS Shifa Karachi, from 26 Sep 2013 to 25 Mar 2014.
Material and Methods: All specimens were inoculated on blood and macConkey agar, incubated aerobically at 35°C - 37°C for 18 to 24 hours. After identification of gram negative rods by colony morphology, Gram's staining and biochemical reactions, these were screened for Carbapenems resistance with imipenem and meropenem 10 µg discs along with routine first and second line antibiotics by Kirby-Bauer disc diffusion technique according to Clinical Laboratory Standard Institute (CLSI) guide lines. All isolated CPE were saved and then inoculated on Mueller-Hinton agar (MHA). Antimicrobial susceptibility against polymyxin B, Tigecycline and Fosfomycin was done by Kirby-Bauer disc diffusion method using disc polymyxin B 300 units, Tigecycline 15µg and Fosfomycin 200µg. Zone diameters greater than 24 mm were taken as sensitive for Tigecycline 15µg, 16mm for Fosfomycin 200 µg and 12 mm for polymyxin B 300 units.
Results: Clinical specimens of 171 patients who fulfilled the inclusion criteria were included in our study. Mean ± SD of age was 42.02 ± 22.367 with C.I (38.65 - 45.40). Out of 171 patients 110 (64%) were male and 61 (36%) were female. In vitro susceptibility results revealed that all the 171 (100%) CPE isolates susceptible to PolymyxinB, while susceptibility against Fosfomycin and Tigecycline was 132 (77%) and 49 (29%) respectively.
Conclusion: CPE were found to be 100% susceptible to polymyxinB, while for Fosfomycin and Tigecycline susceptibility was 77% and 29% respectively.

Keywords: Carbapenemase producing Enterobacteriaceae, Fosfomycin, PolymyxinB, Tigecycline.

INTRODUCTION

Bacteria started to develop resistance against antibiotics immediately after the discovery of antibiotics, through production of different enzymes. These enzymes whether penicillinases or cephalosporinases lead to the development of resistance. Carbapenems were considered the only β-lactam agents active against such extended - spectrum B - lactamase-producing strains[1], but irrational use of Carbapenems has also resulted in the development of resistance to this class of antibiotics as well.

These Carbapenemases are most often carried and expressed by Klebsiella pneumonae, Klebsiella oxytoca, Salmonella enterica, Citrobacter freundi, Enterobacter aerogenes, Enterobacter cloacae, Proteus mirabilis, Serratia marcescens, as well as in nonfermenting Gram-negative bacilli like Pseudomonas aeruginosa, Pseudomonas putida and Acinetobacter baumini spp[1].

Carbapenems were first introduced in 1987. These are the beta lactam antibiotics, derived

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from Thienamycin produced by Streptomyces cattleya. Imipenem and Meropenem are the most widely used Carbapenems, while the other members include Ertapenem, Panipenem, Doripenem and Faropenem. These are the most potent beta lactams with widest spectrum of antibacterial activity. They are effective against gram positive, gram negative and anaerobic bacteria[2].

Unfortunately antimicrobial resistance follows antimicrobial use & first case of Carbapenem resistance was reported in 1996. The Carbapenems resistance in Gram negative rods (GNR) is now a global issue. The prevalence of Carbapenem resistant GNR in USA is about 5.6%, which infact has increased from 0.6% about a decade ago, in India it is about 8%, while in Pakistan the prevalence is 18.5%[3].

Carbapenems are often the antimicrobials of last resort to treat infections due to extended spectrum beta-lactamase (ESBL) or plasmid mediated AmpC (pAmpC) producing organisms of the Enterobacteriaceae family[1]. These pathogens are also resistant to other antibiotic classes including quinolones, aminoglycosides and trimethoprim–sulfamethoxazole and other classes[2].

Infections caused by CPE are serious threat to hospitalized patients. CPE often demonstrate resistance to many other classes of antibiotics thus limiting therapeutic options. Infections with CPE result in poor outcomes and unnecessary financial burden over patients and hospitals. In order to deal with such situation Tigecycline, polymyxins (colistin and polymyxinB) and Fosfomycin are considered possible candidate therapies for infections caused by CPE. According to one of the studies conducted in UK the sensitivity pattern of polymyxinB, Fosfomycin and Tigecycline against CPE was 92%, 60.5% and 46.9% respectively[4]. Rationale of the my study is to find out suitable antimicrobial agents against CPE in order to guide the clinician and other health providers to formulate anti-microbial treatment strategy for such drug resistant organisms.

MATERIAL AND METHODS

This Cross sectional study was carried out in the Department of Microbiology, PNS Shifa Karachi from 26 September 2013 to 26 March 2014. Sample size was calculated by using WHO sample size calculator, taken sensitivity pattern of Tigecycline against CRE is 46.9%, margin of error (d) =7.5%, confidence interval 95%, the estimated sample size was n=171. Sampling technique employed was Non probability consecutive. All CPE detected and confirmed by MODIFIED HODGE TEST (MHT)[5] in the laboratory were included in the study. While all non CPE were excluded from the study. Repeat samples of same patients were excluded too. Permission from institution's ethical review board was obtained prior to study. Hospital identity number, age and gender of the patients were recorded. All specimens were inoculated on blood and macConkey agar incubated aerobically at 35^{o}C for 18 to 24 hours. After identification of gram negative rods by colony morphology, Gram's staining and biochemical reactions, they were screened for CPE with imipenem and mero-penem 10µg discs along with routine first and second line antibiotics by Kirby-Bauer disc diffusion technique according to CLSI5 guide lines. The isolates with zone diameter equal to or less than 23mm against imipenem and meropenem were considered as CPE, as per CLSI guidelines. To control the possibility of observer bias, zones of inhibition were checked by three independent observers and consensus was drawn accordingly. Further confirmation of CPE was done by phenotypic confirmatory test MHT[1]. All isolated CPE were saved and then inoculated on MH agar.

Antimicrobial Susceptibility against poly-myxinB, Tigecycline and Fosfomycin was done by Kirby-Bauer disc diffusion method using disc, Tigecycline 15 µg[6], Fosfomycin 200 µg[7] and polymyxinB 300 units[8].

All the data was entered in a specially designed performa. Since there was no direct involvement of patients being lab specimens, informed consent was not an issue in this study. The data obtained was entered in SPSS-17 for statistical evaluation. Mean and SD was calculated age, frequency and percentage were calculated for gender and outcome variable i.e. sensitivity pattern of PolymyxinB, Fosfomycin and Tigecycline. (sensitive/resistant) effect modifier was control through stratification of age and gender to see the effect of these on outcome variable. Post stratification applying chi square test taken $p \leq 0.05$ as significant.

RESULTS

The totals of 171 patients who fulfilled the inclusion criteria were included in our study. Mean ± SD of age was 42.02 ± 22.367 with C.I (38.65-45.40) years. Out of 171 patients, 110 (64%) were male while remaining 61 (36%) were female.

About 49 CPE isolates (29%) were found susceptible to Tigecycline whereas 122 isolates (71%) were resistant to it. Similarly, 132 CPE isolates (77.2%) were found susceptible to Fosfomycin while 39 isolates (22.8%) were resistant to it. For Polymyxin B, all 171 cases (100%) were found susceptible (table).

Table: Sensitivity pattern of polymyxin B, fosfomycin and tigecycline (n=171).

Group	Antimicrobial susceptibility	
	Sensitive	Resistance
Polymyxin B	171 (100%)	0 (0%)
Fosfomycin	132 (77%)	39 (23%)
Tigecycline	49 (29%)	122 (71%)

DISCUSSION

The emergence of multidrug-resistant (MDR) Gram-negative bacilli creates a significant problem for the treatment of nosocomial infections. Since the Carbapenems remains the strongest class of antibiotic against resistant bacteria, the worldwide spread of CPE isolates represents a serious threat to health care systems all over world[9]. CPE isolates are nonsusceptible in vitro to all β-lactams, including β-lactam/β-lactamase inhibitor combinations, Carbapenems, Fluoroquinolones, and frequently to aminoglycosides. Thus, our therapeutic options against infections due to Carbapenemase producing bacteria are often limited to Tigecycline, Fosfomycin and Colistin.

Susceptibility to polymyxin B amongst clinical CPE isolates ranges globally from 80 to 100%. However, in some areas resistance can be very high due to the clonal spread of resistant strains[4]. Our results have revealed that 100% of CPE isolates were susceptible to colistin. Similar susceptibility results have been reported from Haryana India and Spain with 100% and 97.5% CPE isolates being susceptible respectively[10,11].

Similarly a study done in UK has revealed that 92% of their CPE isolates were susceptible to polymyxin B[4].

The better in vitro efficacy of colistin against CPE in our set up is probably because of restricted usage due to high cost and limited availability of antimicrobial. However it is mandatory that we should use this antimicrobial judicially and with caution, because indiscriminate use of this antimicrobial can result in the emergence of resistance. The in vitro activity of Fosfomycin against CPE isolates in our set up was also very encouraging as 74% of CPE isolates were susceptible to this antimicrobial. Comparable results have been reported from UK in 2011 where 60.5% of their isolates were susceptible to this compound[4]. Another study done in Germany by agar dilution method showed that 72% of their CPE isolates were susceptible to Fosfomycin[12]. The activity of Fosfomycin was evaluated in one of the studies in USA, against 68 KPC producing K. pneumoniae isolates, 23 of which were non-

susceptible to Tigecycline and colistin. The evaluation revealed that susceptibility rates were 93% for the overall group, 87% for the group nonsusceptible to Tigecycline and colistin and 83% for the extremely drug-resistant subgroup that was nonsusceptible to Tigecycline and colistin[1].

Whereas we found only 29% of our CPE isolates to be susceptible to Tigecycline, the results of studies carried out in other parts of the world are different from our finding. A study done in UK showed that 46% of their isolates were susceptible to Tigecycline[4]. In another comparative study done in UK in which samples were collected not only from UK but from India as well, it was found that susceptibility against Tigecycline was 64%, 56% and 67% against CPE isolates from UK, Chennai and Haryana respectively[10].

Our study has showed that both colistin and Fosfomycin have very good potential to be used to treat infections caused by CPE. As regards Tigecycline although results of our study has not been very encouraging but we need to do more large scale studies to find its potential for such isolates. It is imperative that we need to follow strict infection control policies in our health care facilities to limit the spread of infections caused by CPE. It is also incumbent upon microbiologists, clinicians and health care administrators to keep a very close watch on the usage of Carbapenems as well as other antimicrobials to preserve these precious antimicrobials to treat life threatening infections. Combination therapy should be encouraged instead of mono therapy to prevent the development of resistance.

CONCLUSION

CPE isolated from different specimens in our set up were found to be 100% susceptible to polymyxin B, while for Fosfomycin and Tigecycline sensitivity was 77% and 29% respectively. These antimicrobials thus have potential to be used to treat the infections caused by CPE.

CONFLICT OF INTEREST

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

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