ORIGINAL ARTICLE

ACTIVITY OF PIPERACILLIN/TAZOBACTAM AND CEFOPERAZONE/SULBACTAM AGAINST PATHOGENIC ISOLATES OF ENTEROBACTERIACEAE

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ABSTRACT

Background: Enterobacteriaceae are a large family of gram negative bacteria including many genera. The emergence and spread of resistance in Enterobacteriaceae is increasing due to the production of extended spectrum beta lactamase (ESBL) enzymes. Combinations of Beta-lactam/Beta-lactamase inhibitors such as Piperacillin/Tazobactam and Cefoperazone/Sulbactam have good activity against these isolates. The purpose of this study is to determine the antimicrobial susceptibility pattern of Piperacillin/Tazobactam and Cefoperazone/Sulbactam against isolates of Enterobacteriaceae from a tertiary care hospital laboratory of Pakistan. This study validates better options for empirical antimicrobial treatment for this group of difficult to treat pathogens.

Methods: A total of 2111 clinical samples were received for culture and sensitivity from both in and outpatients. Samples were inoculated on Chocolate agar, Sheep blood agar and MacConkey's agar and were incubated for 24 to 48 hours according to standard technique. All isolates belonging to the family Enterobacteriaceae identified by conventional biochemical tests were included in the study. Antimicrobial sensitivities of Enterobacteriaceae were tested and interpreted by Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institution criteria.

Results: Out of 2111 clinical samples, 214 isolates of Enterobacteriaceae were identified. Escherichia coli was recognized as the predominant pathogen (130/214; 60.75%). Frequency of extended spectrum beta lactamases producing Enterobacteriaceaewas100/214(47%) in 214 samples. Antimicrobial sensitivity was 80% (172/214) to Piperacillin/Tazobactam and 83% (178/214) to Cefoperazone/Sulbactam against the total 214 isolates of Enterobacteriaceae.

Conclusion: Piperacillin/Tazobactam and Cefoperazone/Sulbactam are potential and better empirical treatment options for treating isolates of Enterobacteriaceae. This will help in reducing selection pressure on last resort antimicrobials and hence curtail antimicrobial resistance.

KEYWORDS: Antimicrobial susceptibility. Piperacillin/Tazobactam. Cefoperazone/Sulbactam. Enterobacteriaceae. Beta lactamases.

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INTRODUCTION

The family of Enterobacteriaceae includes many genera which are responsible for wide range of infections. They possess a complex antigenic structure, and produce a variety of toxins and enzymes.[1] Antibiotic resistance in isolates of Enterobacteriaceae is emerging in many parts of the world as a major

threat to successful therapy of infection. Beta-lactam antimicrobial agents are the most commonly used for treatment of bacterial infections.[2] Resistance to Beta-lactam antibiotics among isolates of Enterobacteriaceae is most often due to the production of beta-lactamases.[3] These enzymes destroy the beta-lactam ring of the beta-lactam antibiotic.[4] They bind to and prevent the action of penicillin binding protein which are responsible for building and maintenance of peptidoglycan layer.[5,6] The extended spectrum beta lactamases (ESBLs) are commonly inhibited by beta-lactamase inhibitor, such as Clavulanic acid, Sulbactam and Tazobactam.[7] Tazobactam and Sulbactam have been combined with Piperacillin and Cefoperazone respectively to enhance the bactericidal activity.[8] Piperacillin is a semi synthetic Penicillin while Tazobactam is a beta lactamase inhibitor.[9] Combination of Piperacillin with Tazobactam is more effective against Enterobacteriaceae.[10,11,12] On the other hand, Cefoperazone is a semi synthetic cephalosporin while Sulbactam is a good inhibitor of beta lactamase. Combination of Sulbactam with Cefoperazone enhances its bactericidal activity.[13]

This study was done to evaluate the antimicrobial susceptibility pattern of Piperacillin/Tazobactam (Pip/Tazo) and Cefoperazone/Sulbactam (Cef/Sul) against isolates of Enterobacteriaceae from various clinical samples of inpatients and outpatients. The extracted data will be used to guide the physicians towards better antimicrobial options and will reduce antimicrobial resistance.

METHODS

This observational study was carried out in the Clinical Microbiology Laboratory of Dr. Ziauddin University Hospital. The study was conducted over a period of six months from 1st May, 2010 to 30th November, 2010. Specimens including urine, blood, pus aspirates, sputum, tracheal aspirates, catheter tips and body fluids were collected in a sterile leak proof container from both inpatients and outpatients. The samples were initially inoculated on Blood agar, MacConkey's agar and Chocolate agar. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED). Blood culture bottles were kept at 37°C under aerobic condition and then sub-cultured on MacConkey's agar and Chocolate agar. After 24 hours of incubation, suspected colonies of Enterobacteriaceae were screened by gram staining and oxidase test. They were further identified by biochemical tests (i.e. Sulphide Indole motility, Citrate utilization, Urease production, and Triple Sugar Iron tests) with the aid of Analytical Profile Index 20 Enterobacteriaceae (API 20 E).[1]

Antibiotic susceptibility testing of organism was performed on Muller Hinton agar (MHA) by Kirby-Bauer disk diffusion method. The antimicrobial discs, Pip/Tazo (100/10µg) and Cef/Sul (75/30µg) were used. Interpretation of the results was done according to the recommendations of Clinical Laboratory Standard Institution (CLSI) guidelines.[14] American type of Culture collection (ATCC®) Escherichia coli (E. coli) 35218 for Beta-lactam/Beta-lactamase inhibitor combinations and ATCC® E. coli 25922 were used as control strains.

RESULTS

During the period of study a total of 2111 samples were processed, out of which 454 (21%) were culture positive. From these culture positive samples 214 (47%) yielded the growth of Enterobacteriaceae. In this study, E. coli (130/214; 60.75%), was found to be the highest causative organism from isolates of Enterobacteriaceae, followed by Enterobacter (80/214; 37.39%), Klebsiella (03/214; 1.4%), and Citrobacter species (01/214; 0.46%) as shown in Figure 1. In Enterobacteriaceae 47% isolates were found as ESBL producers while 53% were found to be non ESBLs producing organisms **(Figure 2)**. In total, including all isolates of Enterobacteriaceae the antimicrobial sensitivity is 80% (172/214) for Pip/Tazo and 83% (178/214) for Cef/Sul. The sensitivity pattern of non-ESBL and ESBL producing isolates are shown in **Table 1**.

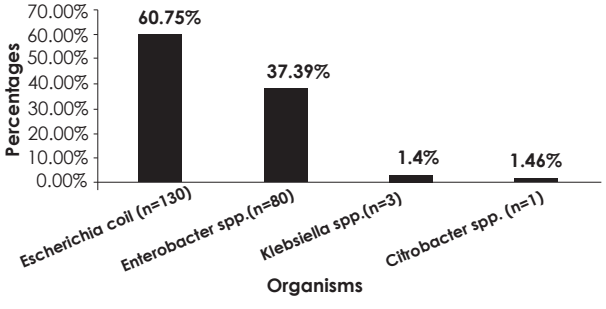

Figure 1: Percentage distribution of isolates of Enterobacteriaceae in different samples (n=214)

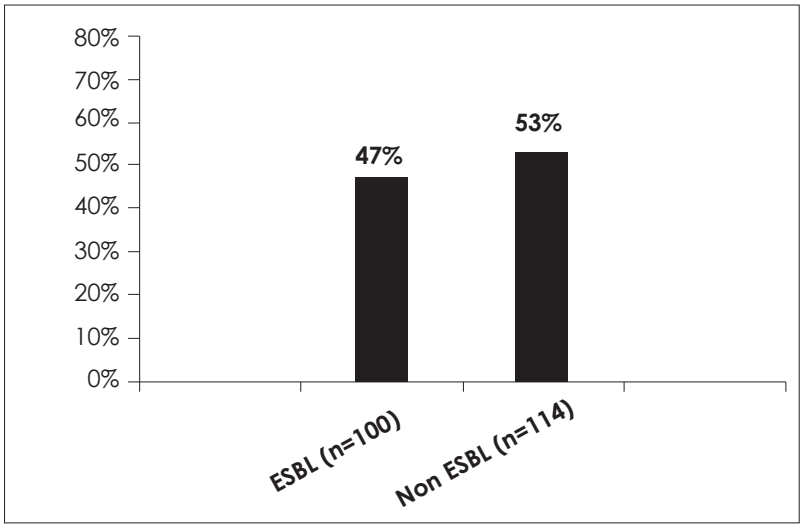

Figure 2: Percentage distribution of ESBL and Non ESBL producing organisms in total number of Isolates of Enterobacteriaceae (n=214

Table 1: Antimicrobial susceptibility pattern of Piperacillin-Tazobactam and Cefoperazone-Sulbactam against Non-ESBL and ESBL producing Enterobacteriaceae (n=214)

Antibiotics	Non ESBLs (n=114)		ESBLs (n=100)	
	Number of isolates (Percentages)		Number of isolates (Percentages)	
	Sensitive	Resistant	Sensitive	Resistant
Piperacillin- Tazobactam	108/114 (95%)	6/114 (6%)	64/100 (64%)	36/100 (36%)
Cefoperazone-Sulbactam	111/114 (97%)	3/114 (3%)	67/100 (67%)	33/100 (33%)

DISCUSSION

Enterobacteriaceae are the most common group of gram negative rods, whose natural habitat is the intestinal tract of human and animals.[15]They have emerged as important nosocomial pathogens, responsible for causing a wide range of infections.[16] The increased incidence of infection due to these organisms is the result of frequent use of broad spectrum Beta-lactam antibiotics.[17,18] The resistance to antimicrobials has increased over the years resulting in increased illness, deaths and health-care costs.[19] The use of broad spectrum Beta-lactams or a combination of Beta-lactamase inhibitor with Beta-lactamase is currently the most successful strategy to combat resistance.[20]

An Indian study reported the sensitivity of 97.2% to Pip/Tazo and 94.6% to Cefo/Sul.[21]A Turkish study also found Cefo/Sul and Pip/Tazo to be very effective against isolates of Enterobacteriaceae.[22]

Prevalence of ESBLs producing strains in various species of Enterobacteriaceae differs in different countries and in different hospitals.[23] Few studies from Pakistan have reported high frequency of ESBLs positivity rate. Shah et al. reported 48%, Zaman et al. reported 35%, while Jabeen et al. reported 40% ESBL isolates of Enterobacteriaceae.[24,25,26]Another study from United Arab Emirates has reported 41% while 64.78% ESBL producing isolates have been reported from India.[27,28] On the other hand the sensitivity to Beta-lactam/Beta-lactamase inhibitor combination is not very good against ESBLs producing organisms as compared to Non ESBLs producing gram negative rods.[29]

CONCLUSION

Pip/Tazo and Cef/Sul are good empirical antimicro-

bial options for treating infections caused by Enterobacteriaceae. This study showed good sensitivity results to Pip/Tazo and Cef/Sul. High prevalence of ESBL production was also noted in the isolates of Enterobacteriaceae with reduced sensitivity pattern of both the antimicrobials. Therefore, it is recommended to treat only non-serious ESBL producing organisms with these antimicrobials. In view of an increasing prevalence of Beta-lactamase producing organisms, this sensitivity pattern will help the physicians in better selection of empirical antimicrobial treatment options for serious infections caused by Enterobacteriaceae.

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