ORIGINAL ARTICLE

Activity of Beta-Lactam Beta-Lactamase Inhibitor Combinations Against Extended Spectrum Beta-Lactamase Producing *Enterobacteriaceae* in Urinary Isolates

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ABSTRACT

Objective: To determine the susceptibility pattern of beta-lactam beta-lactamase inhibitor combinations against extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in urinary isolates.

Study Design: Observational study.

Place and Duration of Study: Ziauddin University Hospital, Karachi, from February to October 2008.

Methodology: A total of 190 consecutive non-duplicate isolates of ESBL producing *Enterobacteriaceae* from urine samples of in-patients were included in the study. Urinary samples from out-patients, repeat samples and non-ESBL producing isolates were excluded. Detection of ESBL was carried out by double disk diffusion technique. Antimicrobial susceptibility testing was performed using modified Kirby Bauer's disk diffusion method according to CLSI guidelines. Statistical analysis was performed by SPSS version 10.

Results: Of the 190 ESBL isolates tested, 88 cases (46.31%) were sensitive and 6 cases (3.15%) were resistant to all three combinations, the rest 96 cases (50.52%) were resistant to at least one of the combinations. Susceptibility pattern of cefoperazone/sulbactam, piperacillin/tazobactam, and amoxicillin/clavulanic acid was 95.26, 92.10, and 44.31 percent respectively.

Conclusion: Cefoperazone/sulbactam exhibited the best activity against ESBL producing *Enterobacteriaceae* followed by piperacillin/tazobactam. Hospital antibiotic policies should be reviewed periodically to reduce the usage of extended spectrum cephalosporins and replace them with beta-lactam beta-lactamase inhibitor combinations agent for treating urinary tract infections.

Key words: *Beta-lactam. Beta-lactamase. Extended-spectrum beta-lactamase. Enterobacteriaceae. Urinary tract infections.*

INTRODUCTION

Antibiotic resistance is widespread and increasing worldwide at an accelerating pace. There is an increased risk of acquiring infection from resistant micro-organism in patients who receive antimicrobials and these infections may lead to increased mortality and morbidity.[1] Widespread use of antibiotics fosters selection of the resistant organisms that rise in prevalence locally and spread worldwide. The emergence of extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* at the end of the 1980s resulted in nosocomial outbreaks and hyperendemic situations in hospitals and long-term care facilities.[2] Afterward, ceftazidime-resistant strains of *Klebsiella pneumoniae (K. pneumoniae)* were isolated in several French hospitals which produce a novel plasmid-encoded ceftazidime-hydrolyzing beta-lactamase.[3] Due to their spectrum of activity against oxyimino-cephalosporins (e.g., cefotaxime and ceftazidime), these enzymes became known as ESBLs.[4]

Patients infected with ESBL producing organisms are at risk for poor outcome if they are treated with antibacterials to which the organisms pose high level resistance. A recent study from Pakistan documented that ESBL producing organisms were most frequently isolated from urine specimen (45.1%).[5] In hospitalized patients with positive cultures for ESBL producing *Escherichia coli (E. coli)*, the majority of instances the isolate attributed to a clinical infection rather than colonization and the commonest clinical specimen to yield the organism were urine, which was positive in 57.8% of patients.[6] Two previous studies from Pakistan reported a frequency of 40% and 35.5% ESBL producing organisms among clinical isolates.[7,8]

Outbreaks of ESBL producing *K. pneumoniae* and *E. coli* infections have been reported with the overuse of expanded-spectrum cephalosporins, especially ceftazidime.[9] Because, ESBLs are inhibited by beta-lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam, beta-lactam beta-lactamase inhibitor combinations has been considered for the treatment of infections due to ESBL producing organisms. Beta-

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lactam beta-lactamase inhibitor combinations indicate potential efficacy in *in vitro* and *in vivo* models.[4]

It is useful to determine important trends and geographical variation of antimicrobial resistance among urinary tract pathogens through worldwide surveillance.[10] The present study was carried out to determine the susceptibility pattern of beta-lactam beta-lactamase inhibitor combinations: cefoperazone/sulbactam, piperacillin/tazobactam, and amoxicillin/clavulanic acid against ESBL producing *Enterobacteriaceae* in urinary isolates of in-patients for guiding the clinicians to decide the better empirical antimicrobial option for treating urinary tract infections (UTIs).

METHODOLOGY

This observational study was conducted over a period of 8 months from February, 2008 to October, 2008 in the Department of Clinical Microbiology of Ziauddin Medical University Hospital, Karachi. A total of 190 non-duplicate consecutively isolated ESBL producing *Enterobacteriaceae* from urine samples of in-patients were included in the study. Urinary samples from out-patients, repeat samples and non-ESBL producing isolates were excluded from the study. Written approval from the institutional ethical committee was obtained. Informed consent was taken from either patient or any other patient's relative.

Urine samples were received in a sterile container supplied from the Microbiology Laboratory. These urine samples were inoculated on Cystein Lactose Electrolyte Deficient medium (Oxoid Ltd., England) and incubated at 37°C in ambient air for 24 hours, using standard microbiological techniques.[11] *Enterobacteriaceae* isolated in urine samples were identified using routine biochemical tests.[11]

Detection of ESBL was carried out by double disk diffusion technique by using disks containing amoxicillin/ clavulanic acid (20/10 µg) placed in the centre of the sensitivity plate with the disks of aztreonam (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), and cefepime (30 µg) that were applied around the amoxicillin/ clavulanic acid disk (20/10 µg) 25-30 mm apart.[12] The plates were incubated overnight at 37°C in an ambient air incubator. After incubation, ESBL production was detected by the presence of expansion, augmentation, or window formation of cephalosporins, or aztreonam disc zone by the clavulanate. At the same time antimicrobial susceptibility testing of amoxicillin/ clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), and cefoperazone/sulbactam (75/30 µg) were performed on Muellar Hinton agar medium (Oxoid Ltd., England) using modified Kirby Bauer's disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.[13] Zone size cut-off of cefoperazone/sulbactam was taken according to Arthur Barry's study.[14] *E. coli* American Type Culture Collection (ATCC®) 35218 and *K. pneumoniae* ATCC® 700603 were used as controls.

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version-10. Frequency and percentages were computed for presentation of all categorical variables like micro-organisms, susceptibility of beta-lactam beta-lactamase inhibitors combinations against ESBL producing *Enterobacteriaceae*, and ESBL positivity.

RESULTS

There were 190 specimens of urine yielding growth of ESBL producing isolates of *Enterobacteriaceae*. In these 190 ESBL isolates; 157/190 isolates of *E. coli*, (82.63%) while only 04/190 isolates of *Proteus mirabilis (P. mirabilis)* [2.10%] showed ESBL positivity as shown in Figure 1.

Overall, cefoperazone/sulbactam exhibited the best activity against ESBL producing *Enterobacteriaceae* followed by piperacillin/tazobactam. Amoxicillin/ clavulanic acid was found to have a poor activity against all ESBL producing *Enterobacteriaceae* as shown in Table I. Only 09 ESBL positive isolates were resistant to cefoperazone/sulbactam, of which 6 isolates were *E. coli* and 03 isolates were *Klebsiella* species. No resistance was observed against cefoperazone/sulbactam in *Enterobacter* species. and *P. mirabilis* group. In piperacillin/tazobactam group, 15 ESBL positive isolates showed resistance, of which 11 isolates were *E. coli* and 4 isolates were *Klebsiella* species. Resistance was also not observed against piperacillin/tazobactam in *Enterobacter* species. and *P. mirabilis* group. Amoxicillin/ clavulanic acid was resistant in 102 ESBL positive isolates which was high. In these resistant isolates the majority were *E. coli* as shown in Table I. In total, 88 ESBL producing *Enterobacteriaceae* (46.31%) were sensitive and 6 cases (3.15%) were resistant to all three

Table I: Susceptibility pattern of amoxicillin/clavulanic acid (A/C), cefoperazone/sulbactam (C/S), and piperacillin/tazobactam (P/T) against ESBL positive organisms.

Organisms	ESBL positive	No. (percent) susceptible to			No. (percent) resistant to		
		A/C	C/S	P/T	A/C	C/S	P/T
E. coli	157	74 (47.13%)	151 (96.17%)	146 (92.99%)	83 (52.86%)	06 (3.82%)	11 (7%)
Klebsiella sp.	22	11 (50%)	19 (86.36%)	18 (81.81%)	11 (50%)	03 (13.63%)	04 (18.18%)
Enterobacter sp.	07	02 (28.57%)	07 (100%)	07 (100%)	05 (71.42%)	00 (0%)	00 (0%)
P. mirabilis	04	01 (25%)	04 (100%)	04 (100%)	03 (75%)	00 (0%)	00 (0%)
Total	190	88 (46.31%)	181 (95.26%)	175 (92.10%)	102 (53.68%)	09 (4.73%)	15 (7.89%)

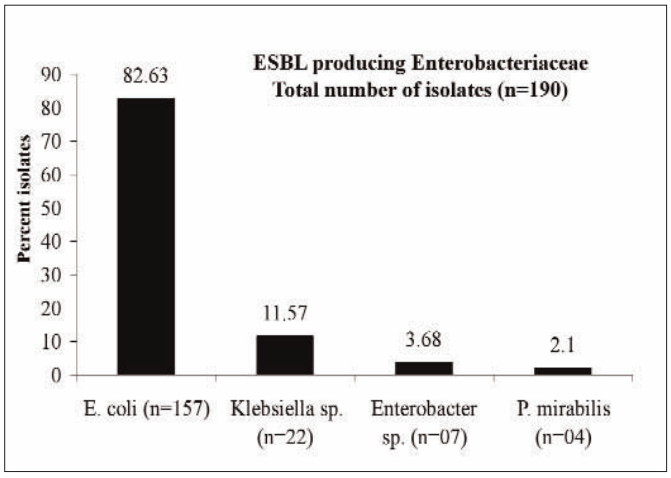

Figure 1: ESBL producing *Enterobacteriaceae*. Percent isolates represent ESBL positive isolates of the individual group divided by total number of ESBL positive *Enterobacteriaceae*.

combinations, while the rest 96 cases (50.52%) were resistant to at least one of the combination.

DISCUSSION

Infections caused by multi-drug resistance gram-negative bacteria especially ESBLs induced infections are recognized worldwide to cause problem in hospitalized patients.[15] Non-judicious and recent use of extended spectrum antimicrobials in hospitals is by far the most important predisposing risk factor to infection with ESBL producing organisms. This vital finding was also noted in this study that the majority of patients were on third generation cephalosporins (cefotaxime or ceftriaxone).

Kanafani *et al.* observed that the most notable risk factor for acquiring infections with ESBL producing organisms was antibiotic consumption within 30 days of the infection, with third generation cephalosporins being associated with the highest risk.[16] Because a wide variety of commonly used antimicrobials (third generation cephalosporins) are resistant to ESBL producing strains, their proliferation poses a serious global health concern that has complicated treatment strategies for a growing number of hospitalized patients.[17] Another concerning factor is the resistance of these ESBL positive organisms to other groups of antimicrobials like quinolones and aminoglycosides allowing very few treatment options.[18]

Beta-lactamase inhibitors usually inhibit ESBLs. There are three beta-lactamase inhibitors (clavulanic acid, tazobactam and sulbactam) are currently in clinical use, and in combination with beta-lactam antibiotics, represent a successful strategy to combat a specific resistance mechanism.[19] With the global issue of ESBL producing organisms, their increasing prevalence and resistance to commonly used antimicrobials especially to treat UTIs. This study's main focus was on the susceptibility pattern of the three beta-lactam beta-lactamase inhibitor combinations.

This study demonstrated that cefoperazone/sulbactam was found to be the best combination followed by piperacillin/tazobactam with the least active combination was amoxicillin/clavulanic acid. Stratchounski *et al.* also reported that among the three beta-lactam beta-lactamase inhibitor combinations tested, cefoperazone/ sulbactam revealed the highest activity against ESBL producing *K. pneumoniae* and *E. coli*.[20] Similar results were reported by McLaughlin *et al.* in which cefoperazone/ sulbactam was proved to be the most efficacious combination followed by piperacillin/ tazobactam and than amoxicillin/clavulanic acid against consecutive isolates of *Enterobacteriaceae*.[21] Ingviya *et al.* observed that among the two combinations, cefoperazone/ sulbactam was more active than amoxicillin/clavulanic acid against ESBL producing *K. pneumoniae* and *E. coli*.[22] Rybak *et al.* reported that piperacillin/tazobactam was found to be slightly more active than cefoperazone/ sulbactam but there was no significance difference among the two combinations with poor activity of amoxicillin/clavulanic acid against ESBL producing *Enterobacteriaceae*.[23] Although all of these studies results indicate almost the same rank of order of activity, difference in the susceptibility rates was observed. The reason could possibly be contributed to the hospital organisms sampled, test methods, site of infection, and the study time interval.

The results of this current study were encouraging with respect to beta-lactam/beta-lactamase inhibitor combinations susceptibilities results, in which very high sensitivity rates to cefoperazone/sulbactam and piperacillin/tazobactam were found as compared to other studies. The low activity of amoxicillin/clavulanic acid was more likely explained by high rates of co-production of ESBL and other plasmid mediated enzymes. This is also likely to be due to the heavy selection pressure from the overuse of this amoxicillin/clavulanic acid combination and seem to be losing the battle. On the other hand, both the relatively high stability of cefoperazone and the increased concentration of sulbactam lead to the greater activity of this combination against ESBL producing organisms.

Currently, carbapenems like meropenem regarded as the drug of choice for serious infections due to ESBL producing organisms like *E. coli* and *K. pneumoniae* and had excellent activity against these organisms.[24] However, for gram negative infections that are not life threatening (like UTIs), carbapenems should not be administered as an empirical therapy because their overuse can pose a significant problem.[25] The emergence of carbapenem resistant organisms such as *Acinetobacter* species, *Stenotrophomonas maltophilia* or *Pseudomonas* species has been associated with the

increase use of carbapenem.[4] Moreover, carbapenem resistance, due to alterations in porin proteins has been observed to develop in *K. pneumoniae*.[4]

A study from a hospital of Taiwan demonstrated that addition of a beta-lactam beta-lactamase inhibitor combination (piperacillin/tazobactam) to the hospital formulary and restriction of third generation cephalosporins (ceftazidime) was associated with a decrease in the percentage of ceftazidime resistant isolates as concomitant decrease was found in the percentage of colonization and infection by ESBL producing *E. coli* or *K. pneumoniae* in patients admitted to the intensive care unit.[9] Beta-lactam beta-lactamase inhibitor combinations like cefoperazone/sulbactam and piperacillin/tazobactam can be useful empiric alternatives for non-serious infections like UTIs caused by ESBL producing organisms. Their substitution in place of third generation cephalosporins (like ceftazidime or ceftriaxone) appears to reduce emergence of the ESBL producing pathogens.

CONCLUSION

Of the currently available beta-lactamase inhibitor combinations, cefoperazone/sulbactam had the best activity against urinary isolates of ESBL producing *Enterobacteriaceae* followed by piperacillin/ tazobactam. Activities of these combining agents need to further evaluate by ascertaining their efficacy in clinical studies. Hospital antibiotic policies should be reviewed periodically to reduce the usage of third generation cephalosporins and replace them with active beta-lactamase inhibitor combinations to decrease the selection pressure and curtail the emergence of antimicrobial resistance.

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