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Original Article

COMPARATIVE *IN VITRO* ACTIVITY OF SUPIME AGAINST GRAM NEGATIVE CLINICAL ISOLATES

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ABSTRACT

Objective: Present investigation was undertaken to know the prevalence of extended spectrum beta-lactamases (ESBLs) among the collected isolates and to analyse the antibiotic susceptibility patterns of cefepime/sulbactam, cefepime/tazobactam, imipenem/cilastatin and cefepime against these isolates.

Methods: A total of 1259 clinical samples were collected from patients suspected of bacterial infection between July 2013 to July 2014. These samples were subjected for bacterial identification. The prevalence of ESBLs among these isolates and antibiotic susceptibility testing were carried out according to the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines (2013).

Results: Out of the samples analyzed, 64.3% (810/1259) samples showed the growth of organisms in the culture medium**.** Of the 810 organisms, 72.7% (589/810) were ESBL positive. Majority of ESBL producing organisms were obtained from urine (32.2 %) followed by blood (28.5 %), swab (12.7%) and sputum (11.3 %). Pus, Bile and fluid samples samples contributed to 8.1 %, 4.0 %, and 3.0% respectively. The organisms that identified were *E. coli* (n=255)*, P. aeruginosa* (208)*, Klebsiella spp.* (81)*, A. baumannii* (32)*,* and *H. Influenzae* (13). Among all drugs tested, cefepime plus sulbactam (Supime) revealed the highest activity against ESBL producing Gram negative organisms. The susceptibility of cefepime plus sulbactam against *E. coli, P. aeruginosa, K. pneumoniae, A. baumannii,* and *H. influenzae* was 89.9%, 84.6%, 85%, 90.4% and 100%, respectively which was high compared to cefepime, cefepime plus tazobactam and imipenem plus cilastatin.

Conclusion: Overall, the results of the present study strongly advocate the superiority of cefepime/sulbactam over cefepime/tazobactam, imipenem/cilastatin and cefepime and can be of very effective alternative to treat against the deadly multidrug resistant Gram negative bacteria.

Keywords: Clinical isolates, Gram-negative, Susceptibility, Supime.

INTRODUCTION

Broad-spectrum cephalosporins are the mainstay in the treatment of various human diseases, such as pneumonia, skin and tissue infections, pelvic inflammatory disease, and other conditions caused by Gram-negative organisms. Cefepime, a fourth generation cephalosporin that has a broader spectrum of activity against Gram negative organisms than other extended-spectrum cephalosporins and also has potential action against Gram positive cocci, such as staphylococcal and streptococcal species [1].

A broad and potent spectrum of activity together with its advanced pharmacological properties makes cefepime a suitable choice of antibiotic for initial empirical therapy for febrile neutropenic patients [1]. The effectiveness of cefepime has been demonstrated by several studies, either alone or in combination [2-4].

However, extensive long term clinical usage lead to the emergence of resistance against this antimicrobial agent [5, 6]. A study done by Jazani *et al.* [7] in which they showed resistance rate of cefepime against *P. aeruginosa* was 75.4%, whereas Satti *et al.* [8] reported that resistance rate of cefepime against *P. aeruginosa* was 71%. A study by Ghafur *et al.* [9] reported 53.8 % of Gram negative organisms were resistant to cefepime. Other *in vitro* studies have recently been published with similar results [10]. Very recently, Chaudhary and Payasi [11] demonstrated that majority of the Gram negative strains were resistant to cefepime (55.4-63.3%).

Resistance to the expanded-spectrum cephalosporins among members of *Enterobacteriaceae*, *Pseudomonas spp.* and *Acinetobacter spp.* may primarily result from extended spectrum β-lactamases (ESBLs) production [12, 13]. The incidence of ESBLs is observed to vary significantly in different geographical areas involving from 73.5 to 66.7% in India [14, 15], 54.7% to 59.2% in Iran [16] and 41% in United Arab Emirates [17].

India has very high rates of ESBLs producing Gram negative organisms thereby leaving carbapenems only reliable options. However, in recent years, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have started showing decreased susceptibility towards carbapenems [18].

The concomitant decreased in efficiency of extended-spectrum cephalosporins emphasize the necessity of selection of an appropriate empiric treatment of nosocomial infections caused by ESBL-producing pathogens. β-Lactam-β-lactamase inhibitor combinations may be considered to be potential alternative to monotherapy of cephalosporins. Keeping it in mind our study was aimed to determine the relative efficiency of cefepime/sulbactam when compared to cefepime, cefepime/tazobactam and imipenem/cilastatin against ESBL-producing Gram negative organisms.

MATERIALS AND METHODS

Drugs

Cefepime plus sulbactam (Supime), cefepime, cefepime+tazobactam and imipenem plus cilastatin were used in the study. All the drugs were reconstituted in water for injection except Supime which was reconstituted in the solvent provided with pack.

Sample collection

Different clinical samples such as blood, pus, sputum, urine, fluid samples, bile, swab, were collected from 1259 (One thousand two hundered and fifty nine) patients suspected of bacterial infection from various hospitals between July 2013 to July 2014.

The collection and processing of the samples were done according to a common standard operating procedure (SOP). The ethical approval of the study is 14-17.

Isolation and identification of pathogens

All the samples were collected asceptically in sterile containers. Urine samples collected in sterile universal container and were directly inoculated onto the cystine lactose electrolyte deficient (CLED) medium. Other specimens involving pus, sputum, bile and fluid samples, collected in sufficient amount and were inoculated on the different non-selective and selective culture media as per the standard microbiological techniques. Blood samples collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37 °C and then subcultured on to the non-selective and selective culture media. The organisms were identified on the basis of colony morphology, Gram staining, motility, and biochemical reactions [19]. Following various selective culture media were used for isolation of different pathogens i.e. for *E. coli* eosine methylene blue (EMB) agar medium was used, for *A. baumannii* leeds *Acinetobacter* agar base medium was used, for *Klebsiella spp.* and *H. influenzae* hicrome *Klebsiella* selective agar base medium and BD brain heart infusion agar with 15% horse blood and bacitracin were used respectively, whereas for *P. aeruginosa* citrimide agar was used

Screening of isolates for ESBL production

Screening of isolates for extended-spectrum beta-lactamases (ESBLs) production was performed according to the procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines [20], using indicator cephalosporins, ceftriaxone (30µg), ceftazidime (30µg) and cefotaxime (30µg). Isolates exhibiting zone size ≤ 25 with ceftriaxone, ≤ 22 for ceftazidime and ≤27 with cefotaxime were considered possible ESBLs producer.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by the cup-plate agar diffusion method; a modification described earlier [21]**.** Inoculum containing 10^6cfu*/ml* of test strain was spread with a sterile swab on a petri dish containing Mueller-Hinton agar and the plates were dried. The cups were made in the agar plate using a sterile cork borer (6.5 mm) and the disks were removed. Then, 30 µl of the antibiotic preparation was placed in the wells using a micro-pipette and allowed to diffuse at room temperature. The plates were incubated in the upright position at 37 °C for 18 hours. After incubation, the zone of inhibition around the wells was measured in mm (millimeter), averaged and the mean values were recorded. Sensitivity of isolated organisms against antibiotics were reported as sensitive (S) or resistant (R) based on the breakpoints.

RESULTS AND DISCUSSION

A total 1259 different clinical samples of urine, blood, pus, sputum, bile, swab and fluid samples were collected from patients admitted to different hospitals of India and these samples were processed for isolation of pathogenic organisms. Out of the samples analyzed, 64.3 % (810/1259) samples showed the growth of organisms while in 449 samples showed no growth in the culture medium (table 1). Of the 810 organisms, 72.7 % (589/810) were ESBL positive. Among the ESBL producing organisms around 32.2 % pathogens were obtained from urine followed by blood, swab and sputum samples which contributed to 28.5 %, 12.7% and 11.3 % respectively. Pus, bile and fluid samples contributed to 8.1 %, 4.0 %, and 3.0 % respectively (table 1).

Table 1: Clinical samples used as a source of the pathogenic isolates and their identification of ESBL positive isolates

Clinical samples	Total clinical specimens	Samples showing growth	ESBL positive isolates	Samples not showing growth
Urine	327	235	190	92
Blood	374	223	168	151
Sputum	150	110	67	40
Swab	139	109	75	30
Pus	119	60	48	59
Bile	104	45	23	59
Fluid samples	46	28	18	18
Total	1259	810	589	449

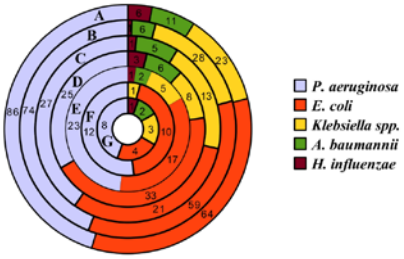
Morphological and biochemical characterization of the ESBL positive pathogens revealed the presence of 5 different Gram negative organisms in clinical samples. The detailed profile of various organisms is shown in fig. 1. The identified bacteria include *E. coli* (n=255), *P. aeruginosa* (208), *Klebsiella spp.* (81), *A. baumannii* (32), and *H. influenzae* (13), indicating *E. coli* (43.3%) was the most dominant pathogen which is in agreement with previous study [22] fig. 2.

In another study, Shafiyabi *et al.* [23], demonstrated 39.6% prevalence of *E. coli*. This goes with results that obtained in Tanzania [24]. In our study, *P. aeruginosa* contributed 35.3% to the isolated pool of organisms. A study performed by Rit *et al.* [25] reported 50.2% prevalence rate of *P. aeruginosa*. Other studies reported 10 to 60 % incidence of *P. aeruginosa* in various clinical samples [26-28].

The results of the present study showed that the prevalence of *Klebsiella spp.* was 13.7 % which were in accordance with the results reported by Kumar and Kalpana [29], where they demonstrated prevalence of *K. pneumoniae* (14.5 %) among clinical isolates.

However, *A. baumannii* (5.4 %) contributed less in the present study which was in agreement with previous studies [30, 31]. *E. coli* was the most prevalent pathogen contributing 34.0 %, 29.0 %, 10.6 %, 9.8 %, 9.0 %, 4.6 %, and 3.0 % in urine, blood, sputum, swab, pus, bile and fluid samples, respectively. Prevalence of other ESBL producing organisms among various specimens is depicted in fig. 3.

Resistance to antibiotics is a significant problem in the treatment of serious nosocomial infections. Antibiotic therapy is often empiric, until a specific pathogen and its antibiotic susceptibility is known. The third-generation cephalosporins are widely used for empiric therapy but their effectiveness has been limited by the increasing prevalence of ESBL producing strains of Pseudomonas spp. and *Enterobacteriaceae* that produces mainly β-lactamases [32].



A-Urine; B-Blood; C-Sputum; D-Swab; E-Pus; F-Bile; G-fluid samples

Fig. 1: Profile of different clinical isolates isolated from various samples

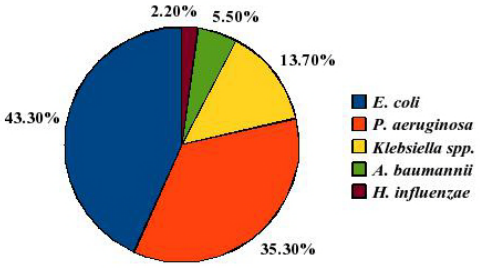


Fig. 2: Prevalence of various pathogens

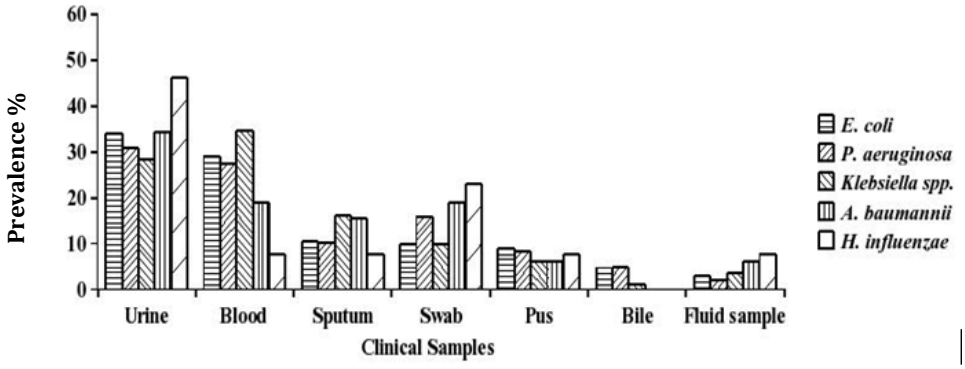


Fig. 3: Prevalence of ESBL positive pathogens

Antibiogram profile for all organisms isolated from various clinical samples is presented in fig. 4 and 5. In this study we observed that the activity of cefepime was evidently poor as compared to the other antibiotics, whereas the susceptibility of cefepime plus sulbactam was the highest among all the isolated pathogen. The susceptibility of cefepime plus sulbactam against *E. coli, P. aeruginosa, K. pneumoniae, A. baumannii,* and *H. influenzae* was 89.9%, 84.6%, 85%, 90.4% and 100%, respectively which was high compared to

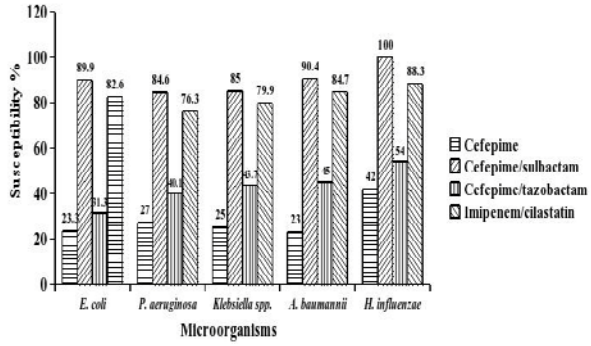


Fig. 4: Susceptibility pattern of Gram negative pathogens isolated

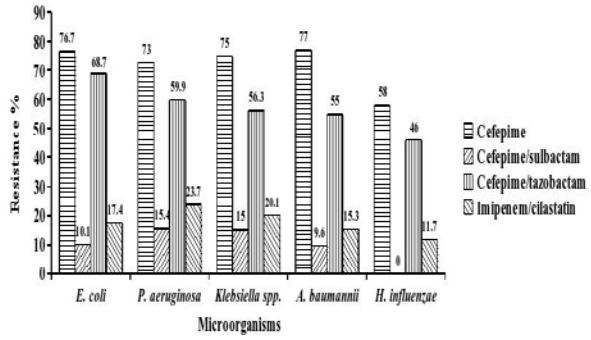


Fig. 5: Resistance pattern of Gram negative pathogens isolated

cefepime, cefepime plus tazobactam and imipenem plus clistatin. Wahid *et al.* [33] reported that cefepime plus sulbactam combination shows 90.0 % sensitivity for *P. aeruginosa*, 90.9 % for *E. coli* and 100% sensitivity for *Klebsiella spp.*

Another study showed that addition of beta lactamase inhibitors drastically reduced MIC of cefepime against ESBL producing bacteria [34].

A high rate of resistance to cefepime (58 %-76.7 %) and cefepime plus tazobactam (46 %-68.7%) was observed. Khalili *et al.* [35] noted the resistance rate of Gram negative bacilli to cefepime 60, 67.9, 37.9 and 50 % in 2007, 2008, 2009 and 2010, respectively in Iran. In another study, resistance to cefepime for *E. coli*, *Klebsiella* and *Pseudomonas* to cefepime was reported to be 65.1, 32.2 and 80 %, respectively [36]. Jazani *et al.* [37] documented 62.4 to 88.4 % burn isolates of *P. aeruginosa* were resistant to cefepime. De Macedo and Santos [38] reported 51.1 % resistance to cefepime for *P. aeruginosa* burn isolates. Endimiani *et al.* [39] noticed that approximately 10-35 % of *P. aeruginosa* clinical isolates were resistant to cefepime in North America, America and Europe.

Ghafur *et al.* [9] reported that addition of tazobactam increased the susceptibility of cefepime from 34.4 to 87.9 % in *E. coli*, from 42.3 to 81.0 % in *Klebsiella spp.* from 72.0 to 81.4 % in *Pseudomonas spp.* and from 17.2 to 54.5 % in *Acinetobacter spp*. Cefepime/tazobactam provided a better invitro sensitivity profile when compared to cefepime alone. Contrary to this, our data showed 54 to 31.3 % susceptibility of cefepime plus tazobactam against various gram negative organisms.

In the present study, incidence of resistance to imipenem plus cilastatin was 11.7 %-23.7 % (table 2). Previous studies showed imipenem plus cilastatin resistance varied from 48.6 to 59.2 % [17, 40].

CONCLUSION

The bacterial susceptibility and profile of all isolates in this study have shown that cefepime/sulbactam and imipenem+cilastatin remain the most effective drugs against Gram negative pathogens, suggesting that use of cefepime/sulbactam over other antibiotics should be preferred. However there is a need to emphasize on the

rational use of antimicrobials and strictly adhere to the concept of reserve drugs to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

CONFLICT OF INTERESTS

Declared None

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