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Research Article

IN VITRO ACTIVITY OF CEFTAZIDIME/SULBACTAM AND CEFEPIME/ SULBACTAM IN COMBINATION AGAINST ESBL PRODUCING ISOLATES

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ABSTRACT

The use of the β-lactamase inhibitors in combination with the β-lactam antibiotics is currently the most successful strategy used for circumventing the resistance mechanisms. The aim of the study was to compare the in–vitro activity of ceftazidime/sulbactam and cefepime/sulbactam in combination against ESBL producing gram negative isolates. In the present study all specimens were cultured and isolates were identified by standard methods. The antimicrobial sensitivity testing was done according to CLSI guidelines by Kirby Bauer's disc diffusion method. The breaking point for antimicrobial sensitivity testing interpretation of these combinations was provided by Venus Research Center. All readings were noted according to reference ranges provided by manufacturer for both the antibiotic combinations.

Keywords: Gram negative bacilli, Ceftazidime, Cefepime, Sulbactam, Kirby Bauer's disc.

INTRODUCTION

Gram negative bacilli, continues to be an important cause of health care associated infections[1]. Treatment in India is based on empirical therapy [2]. These microorganisms have acquired a wide variety of mechanisms to resist the action of antibiotics[3]. Secretion of Extended Spectrum β -Lactamase (ESBL) enzymes is one of the key resistance mechanisms of bacterial drug resistance[2].

The ESBL producing pathogens has become a cause of grave concern today, while dealing with infections in community, nosocomial infections or infections in critically ill patients[2]. Such a wide spread dissemination of bacterial drug resistance to a variety of β -lactam antibiotics possess a serious threat to effective use of these antimicrobial agents[2].

ESBL enzymes are plasmid mediated enzymes & result due to mutation of TEM-1, TEM-2, & SHV-1[2 4].

The efficacy of older broad spectrum antibiotics has been questioned with the observed advent of longer resolution times of infections, treatment relapse & treatment failure resulting in an increased morbidity & mortality[2].

The third generation cephalosporin like ceftazidime are considered good in therapeutic conditions as they are fairly safe agents for treatment of many serious infections[5].

A forth generation cephalosporin, cefepime, is bactericidal in its action with extended spectrum activity against Gram negative as well as Gram positive pathogens. Cefepime is approved for treating serious infections like pneumonia, uncomplicated & complicated urinary tract infections, skin & soft tissue infections, intra-abdominal infections & febrile neutropenia[6].

It is a current trend to use β-lactam antibiotics in combination with β -lactamase inhibitors such as Sulbactam[7,8].

Sulbactam is competitive irreversible β -lactamase inhibitor & has good inhibitor activity against the clinically important plasmid mediated β-lactamase & most frequently responsible for transferred drug resistance [9]. Sulbactam is approved in many countries including India, to be combined with β -lactam antibiotics[10,11].

Choosing cephalosporin like ceftazidime and cefepime combination with sulbactam, a potent β -lactamase inhibitor would be a strong basis of rational therapeutics.

Hence the present study was carried out to observe the In vitro activity of ceftazidime/sulbactam & cefepime/sulbactam against ESBL producing Gram negative isolates.

MATERIALS AND METHODS

This study was carried out at Pad. Dr. Vitthalrao Vikhe Patil Foundation's Dr. Vitthalrao Vikhe Patil Pharmacy College, Ahmednagar, Maharashtra. From the period of October 2012 to January 2013.

INCLUSION CRITERIA:

For this study samples were selected randomly & multiple but different isolates from single specimen were also considered.

Table 1, shows the distribution of randomly selected clinical specimens including, Gram positive and Gram negative isolates.

Table 1: Distribution of the clinical specimens

Sr. No.	Nature of specimens	Total number of clinical specimens (N)	Growth occurred in total number of clinical specimens (N) (%)
1	Sputum	28	11 (5.98 %)
2	Fluid	28	12 (6.52 %)
3	Blood	27	03 (1.63 %)
4	Urine	45	16 (8.70 %)
5	Pus	58	30 (16.30 %)
Total		**184 (100%)**	**72 (39.13 %)**

METHODOLOGY OF TESTING

All specimens were cultured & isolates were identified by standard methods[12]. Isolates producing Extended Spectrum β - lactamase enzyme (ESBL) were detected by using ceftazidime & ceftazidime clavulanic acid discs as par CLSI guidelines[13].
The antimicrobial sensitivity testing was done according to CLSI guidelines by Kirby Bauer's disc diffusion method. ceftazidime/sulbactam & cefepime/sulbactam combination discs were provided by Venus Medicine Research Center, Venus Remedies Ltd. Baddi (H.P.), to our laboratory for testing. These discs were manufactured by Hi-Media Laboratories, Mumbai as per CLSI standard parameters.
Each disc of ceftazidime/sulbactam & cefepime/sulbactam contains 30 µg of respective antibiotics.
The breakpoints for antimicrobial sensitivity testing interpretation of these combinations were provided by Venus Research Center.
All the isolates were lawn cultured using inoculums matched with Mac-Farland's standard tube No. 5, for turbidity. Mueller Hinton Agar plates were used for antimicrobial sensitivity testing. All plates were kept for incubation at 37^0C, for 18 hrs. ATCC *E. coli* strain No. 35218 & ATCC *Pseudomonas aeruginosa* strain No. 27853 was used as internal quality control stains. After the incubation period was completed all plates were examined & zone of inhibition readings were taken under three headings.

1. Sensitive (Showing wide zone of inhibition of growth - sensitivity).
2. Intermediate sensitive (Showing narrow zone of inhibition of growth - intermediate sensitivity).
3. Resistant (Showing no zone of inhibition of growth up to the margin of disc – resistance).

For convenience intermediate sensitive strains were included into resistant strains.
All readings were noted according to reference ranges provided by manufactures for both the antibiotic combinations.

RESULTS

Table 2: Number of Gram negative bacilli isolated from clinical specimens

Sr. No.	Isolates	Blood (N =03)	Urine (N=16)	Sputum (N=11)	Fluid (N=12)	Pus (N=30)	Total (N=72)
1.	*Pseudomonas aeruginosa*	00	02	03	04	07	16
2.	*Non- fermenter*	00	01	00	00	00	01
3.	*Acinetobacter species*	00	00	00	02	01	03
4.	*Escherichia coli*	00	04	01	04	09	18
5.	*Klebsiella pneumoniae*	01	04	04	02	07	18
6.	*Proteus mirabilis*	00	01	00	00	01	02
7.	*Proteus vulgaris*	00	01	00	00	00	01
8.	*Citrobacter freundii*	00	01	00	00	02	03
Total		**01 (1.61%)**	**14 (22.58%)**	**08 (12.90%)**	**12 (19.35%)**	**27 (43.55%)**	**62 (100%)**

Table 3: ESBL producing Gram negative isolates

Sr. No.	Isolates	Total number of isolates	Total number of isolates detected as ESBL enzyme producers
1.	*Pseudomonas aeruginosa*	16	10
2.	*Non-fermenter*	01	00
3.	*Acinetobacter species*	03	01
4.	*Escherichia coli*	18	11
5.	*Klebsiella pneumoniae*	18	11
6.	*Proteus mirabilis*	02	00
7.	*Proteus vulgaris*	01	01
8.	*Citrobacter freundii*	03	01
Total		**62 (100 %)**	**35 (56.45 %)**

Table 4: Antimicrobial sensitivity test results of isolates for Ceftazidime/Sulbactam combination

Sr. No.	Isolates	Number of strains producing ESBL (n)	Number of strains sensitive (S)	Number of strains resistant (R)
1	*Pseudomonas aeruginosa*	10	09	01
2	*Acinetobacter species*	01	00	01
3	*Escherichia coli*	11	06	05
4	*Klebsiella pneumoniae*	11	07	04
5	*Proteus vulgaris*	01	00	01
6	*Citrobacter freundii*	01	01	00
Total		**35 (100 %)**	**23 (65.71 %)**	**12 (34.29 %)**

Table 5: Antimicrobial sensitivity test result of isolates for Cefepime/Sulbactam combination

Sr. No	Isolates	No of Strains producing ESBL	No of strains Sensitive (S)	No of Strains Resistant (R)
01	*Pseudomonas aeruginosa*	10	9	1
02	*Acinelbacter spp*	01	0	1
03	*Escherichia coli*	11	11	1
04	*Klebsiella Pneumoniae*	11	11	0
05	*Proteus vulgaris*	1	0	1
06	*Citrobacter freundii*	01	01	0
Total		**35 (100 %)**	**31 (88.57 %)**	**4 (11.43 %)**

DISCUSSION

Resistance to third and fourth generation cephalosporins has become a major concern worldwide. The use of broad spectrum β–lactam or a combination of β–lactamase inhibitor with β-lactam is currently the most successful strategy to combat resistance. Extended spectrum β–lactamases (ESBL) are plasmid mediated enzymes capable of hydrolyzing and inactivating a wide variety of β–lactam antibiotics. The β–lactamase inhibitors are capable of inhibiting a variety of β–lactamase including ESBL enzyme.

In the present study a total of out of 62 (100%) isolates of Gram negative bacilli (Table 2), 35 (56.45%) were ESBL producing (Table 3). The antimicrobial sensitivity test result of isolates for ceftazidime/sulbactam combination shows encouraging results. *Pseudomonas aeruginosa* 90.00 % of strains producing ESBL were sensitive, whereas for *E.coli* 54.55 % were sensitive. For *Klebsiella pneumoniae* 63.63 % were sensitive. *Citrobacter freundii* showed 100% sensitivity. The antimicrobial sensitivity test result of isolates for cefepime/sulbactam combination shows 90.00 % sensitivity for *Pseudomonas aeruginosa*, for *E. coli* 90.90 % were sensitive whereas, for *Klebsiella pneumoniae* & *Citrobacter freundii* isolates were 100 % sensitive. The study shows that the combination of cefepime/sulbactam shows improved efficacy as compared to the combination of ceftazidime/sulbactam against ESBL producing Gram negative isolates.

CONCLUSION

To conclude, the ESBL isolates of Gram negative bacilli showed good sensitivity pattern in-vitro as compared to resistance. This inspires to carry out in-vivo activity of these combinations & also perform more extensive studies on such

combinations. which shall recommend the clinical utility of these combinations.

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