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Comparative in Vitro Activity of Zydotum against Gram Negative and Gram Positive Clinical Isolates

Manu Chaudhary and Anurag Payasi*
Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, H.P. - 173205 India

Abstract: *The present study was carried out to evaluate and compare the in vitro activity of BL BLI combinations containing sulbactam and tazobactam. Drugs evaluated include ceftazidime+sulbactam (Zydotum) ceftazidime+tazobactam (Combitaz), cefoperazone+sulbactam (Sulprazon), pipercillin+tazobactam (Tazocin) and ceftazidime (C-ZID) alone against gram-negative organisms obtained from different clinical specimens from different hospitals located in North and West region of India. Among 618 samples collected, 238 were sterile and only 380 samples showed the presence of bacterial infections with P. aerugenosa as the most predominant pathogen (56.3%) followed by E coli. (13.4%), S. aureus (10.8%), S. pneumoniae (9.2%,), P. vulgaris (4.7%), H. Influenzae (3.1%) and S. pyogenes (2.4%). Our results showed that the susceptibility of ceftazidime+sulbactam was the highest among all the isolated pathogen; P. aeruginosa (93.4%), E. coli (86.3%), H. influenzae (91.6%), P. vulgaris (83.3%), S. aureus (90.2%), S. pyogenes (88.8%) and S. pneumoniae (82.8%). Pipercillin+tazobactum was the second most effective drug against these gram negative and gram positive pathogens (66.8 to 77.7%), followed by ceftazidime+tazobactam (64.7 to 72.2%), cefoperazone+sulbactam (46.3-72.8%) and ceftazidime alone (25 to 28.6%). The results of the present study advocates the superiority of Zydotum over other tested antibiotics for the treatement of infections caused by ceftazidime resistant gram negative and positive bacteria.*

Keywords: *Clinical isolates, Gram-negative, Gram-positive, Susceptibility, Zydotum.*

I. Introduction

Hospital aquired infections (HAI) are an important public health problem in developing countries as well as in devel-oped ones. HAIs are known to be a major cause of high morbidity and mortality in hospitalized patients.[1] Prevalence of HAI ranges from 3.8% to 18.6% depending on the population surveyed and the definitions used.[2]

It is estimated that 80% of all hospital deaths are directly or indirectly related to HAIs.[3] HAI are most commonly associated with lower respiratory tract infections, urinary tract infections, pneumonia, wound infections, bloodstream infections, surgical site infection (SSI) and sepsis which are primarily caused by a range of gram-negative organisms particularly *E.coli*, *Acinetobacter spp*, *Klebsiella spp*., *Pseudomonas spp.*, Enterobacter spp.[4-9]

These Gram-negative organisms are of particular concern with reported increasing rates of drug resistance.[10-12] Among the β-lactams, third generation cephalosporins, such as ceftazidime, cefotaxime, and ceftriaxone are routinely used in our clinical setting because of their broad-spectrum activity, well-characterized pharmacokinetic and pharmacodynamic properties, and proven safety and efficacy.[13] Ceftazidime was introduced into clinical use in the 1980s because of its broad-spectrum activity against Gram-positive cocci and Gram-negative bacilli, including *Pseudomonas aeruginosa*. Unfortunately, over time, the utility of ceftazidime to treat infections caused by Gram-negative have become compromised by increasing occurence of extended-spectrum β-lactamases (ESBLs)[14], low permeability and overexpression of efflux pumps and biofilm formation.[15]

A number of surveillance reports from across globe indicated the increasing resistance to ceftazidime which ranging 30 to 71 %. [11,16-19] With the drying pipeline of new antimicrobial agents, treatment of Gram negative organisms continues to rely on the theoretical advantages of combination therapy implying that combination drug therapy should be reinforced. In recent years, combination of β-lactam antibiotics with β -lactamase inhibitors such as Sulbactam has been using widely.[20] Sulbactam is competitive irreversible β-lactamase inhibitor and has good inhibitor activity against the clinically important plasmid mediated β-lactamase and most frequently responsible for transferred drug resistance.[21] .Sulbactam is approved in many countries including India, to be combined with β -lactam antibiotics.[20]

Therefore the present study was undertaken to evaluate the in vitro activity of ceftazidime+sulbactam, ceftazidime+tazobactam, cefoperazone+sulbactam, pipercillin+tazobactam and ceftazidime alone against gram-negative organisms obtained from different clinical specimens.

II. Materials And Methods

2.1. Isolation and identification of clinical isolates

A total of 618 clinical specimens consisting of blood (n=268), sputum (205), swab (145) were collected

from different centres of India. The study was conducted between the period January 2014 to February 2015. The identification of all isolates was performed using conventional methods.[22] The collection and processing of the samples were done using a common SOP by all laboratories. All the samples were collected aseptically in sterile containers. Urine samples collected in sterile universal container were directly inoculated to the respective selective media. Other liquid specimens were collected in sufficient amount and inoculated on the different selective and non-selective culture media as per the standard microbiological techniques. For the processing of blood samples, specimens were collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) and were incubated overnight at 37°C. After incubation, subcultured on to the selective and non-selective media. All the media were incubated aerobically overnight at 37?C and the identification of bacteria were performed using standard methodology.[22]

2.2. Antibiotics

Ceftazidime+sulbactam (Zydotum, Venus Remedies Limited, Baddi, Himachal Pradesh, India), ceftazidime+tazobactam (Combitaz, Lupin Laboratories), cefoperazone+sulbactam (Sulprazon, GLS Pharma Limited), pipercillin+tazobactam (Tazocin, Wyeth Pharmaceuticals) and ceftazidime (C-ZID, Emcure Pharmaceuticals) were used in the study. All the drugs except Zydotum which was reconstituted with solvent provided with pack were reconstituted in water for injection prior to use. Working solutions were prepared using MH broth (Mueller Hinton, Himedia, Mumbai, India).

2.3. Antimicrobial susceptibility test by cup-plate method

The cup-plate agar diffusion method, a modification described earlier[23], was adopted to assess the antimicrobial susceptibility of the test solutions. The test organism was picked up with a sterile loop, suspended in Mueller-Hinton broth and incubated at 37°C for 2 h. The turbidity of the suspension was adjusted to 0.5 McFarland's standard (1.5 x 10^8 CFU/mL). Inoculum containing 10^8 CFU/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 (6.5mm). 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 37^0C for 18 hours. After incubation the zone of inhibition around the wells was measured in mm (millimeter), averaged and the mean values were recorded.

III. Results And Discussion

During the study, a total of 618 clinical samples were collected and subjected for isolation of bacteria. Of the collected samples, 380 (61.4 %) samples yielded significant growth and remaining 238 (38.5 %) samples were either sterile or showed no significant growth. Highest number of bacteria were isolated from Blood samples 49.5 % (188/380) followed by swab 29.7 % (113/380), sputum 20.8% (79/380) (Table 1).

Morphological and biochemical characterization of bacteria showed the presence of seven different types of gram positive and gram negative bacteria across all the samples. Among the isolates *P. aeruginosa* 56.3 % (214/380) was the most predominant pathogen followed by E coli 13.4 % (51/380), *S. aureus* 10.8% (41/380), *S. pneumoniae* 9.2% (35/380), *P. vulgaris* 4.7% (18/380), *H. Influenzae* 3.1 % (12/380) and *S. pyogenes* 2.4% (9/380) (Table 2).

The susceptibility of various microorganisms to different drugs is shown in Table 3. Ceftazidime+ sulbactam (Zydotum) emerged as the most efficacious antibacterial agents against all the tested pathogens. For, *P. aeruginosa* 93.4 % isolates were susceptible to ceftazidime+sulbactam against 66.8 % to piperacillin+tazobactam, 65.4 % isolates to ceftazidime+tazobactam and only <47 % isolates were susceptible to ceftazidime alone and cefoperazone+sulbactam. In the case of *E. coli* isolates, susceptibility to various antibacterial agents was 86.3% to ceftazidime+sulbactam, 70.6 % to piperacillin plus tazobactam, 64.7% to ceftazidime+tazobactam, 51 % to cefoperazone+sulbactam and 27.5 % to ceftazidime. Among *H. Influenzae*, susceptibility to various antibacterial agents was 91.6 % to ceftazidime+sulbactam, 75 % to piperacillin plus tazobactam, 66.6% to ceftazidime+tazobactam, 50 % to cefoperazone+sulbactam and 25 % to ceftazidime. *P. vulgaris* isolates susceptibility 83.3% to ceftazidime+sulbactam, 77.7 % to piperacillin plus tazobactam, 72.2% to ceftazidime+tazobactam, 55.5 % to cefoperazone+sulbactam and 27.7 % to ceftazidime. In the case of gram-positive organisms (*S. aureus, S. pyogenes* and *S. pneumoniae*), susceptibility to various antibacterial agents was 82.8-90.2 % to ceftazidime+sulbactam, 73.2-85.7 to piperacillin plus tazobactam, 65.8-68.6% to ceftazidime+tazobactam, 53.6-62.8 % to cefoperazone+sulbactam and 22.2-28.6 % to ceftazidime.

Our data showed the higher susceptibility of ceftazidime+sulbactam against all the isolates which might be due to sulbactam significantly potentiates ceftazidime against both gram negative and positive isolates when compared with piperacillin-tazobactam, cefoperazone-sulbactam and ceftazidime-tazobactam. Wahid et al.[20] also demonstrated that ceftazidime/sulbactam combination works synergistically against *P. aeruginosa*

and E.coli. Zhang and Li[24] showed that combination of sulbactam and ceftazidime at the ratio of 1:1, ceftazidime resistant isolates became susceptible to it. Kolayl et al. [25] reported that ceftazidime + sulbactam may be a resonable alternative to carbapenems in the empirical regimen and is more active than piperacillin/tazobactam and cefoperazone/sulbactam.

Our data demonstrated the least susceptibility of ceftazidime alone against the tested isolates which is in agreement with earlier study where decreased susceptibility of third-generation cephalosporins to *Pseudomonas spp.* and Enterobacteriaceae has been reported mainly due to β-lactamases.[26] Kumar et al.[27] also documented low susceptibility of ceftazidime (35.55%), when compared to piperacillin/tazobactam (87.22%) and cefoperazone/sulbactam (76.67%) in E.coli. Numerous studies from India have shown piperacillin-tazobactam to be better than cefoperazone-sulbactam especially against Pseudomonas sp, Klebsiella sp, and E. coli.[28-30]

IV. Conclusion

From the above data, it is evident that ceftazidime alone is loosing efficacy due to resistant strains and there is need to use combination of BL and BLIs. Further, ceftazidime+sulbactam (Zydotum) has enhanced in vitro antibacterial activity compared to ceftazidime+tazobactam, cefoperazone+sulbactam and piperacillin+tazobactam. Therefore, it is concluded that ceftazidime+sulbactam can one of the best options for the treatment of infections caused by ceftazidime resistant organisms.

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Table 1: A profile of clinical samples used as a source of the pathogenic isolates

Clinical samples	Total clinical specimens	Samples showing growth	Samples not showing growth
Blood	268	188	80
Sputum	205	79	126
Swab	145	113	32
Total	618	380	238

Table 2. Distribution of pathogens among clinical samples.

Clinical specimens	Total no. of isolates collected from various specimens	P. aeruginosa	E. coli	H. influenzae	P. vulgaris	S. aureus	S. pyogenes	S. pneumoniae
Blood	188	112	31	9	5	16	--	15
Sputum	79	36	20	3	-	-	-	20
Swab	113	66	-	-	13	25	9	-
Total	380	214	51	12	18	41	9	35

Table 3: Antibiogram of clinical isolates.

Name of antibiotic	*P. aeruginosa* (n=214)		*E. coli* (n=51)		*H. influenzae* (12)		*P. vulgaris* (18)		*S. aureus* (41)		*S. pyogenes* (9)		*S. pneumoniae* (35)	
	Susceptibility (%)													
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Ceftazidime+sulbactam (Zydotum)	93.4	6.5	86.3	13.7	91.6	8.3	83.3	16.6	90.2	9.7	88.8	11.1	82.8	17.2
Ceftazidime+tazobactam	65.4	34.6	64.7	35.3	66.6	33.3	72.2	27.7	65.8	34.1	66.6	33.3	68.6	31.4
Ceftazidime	27.1	72.9	27.5	72.5	25	75	27.7	72.2	26.8	73.2	22.2	77.7	28.6	71.4
Cefoperazone+sulbactam	46.3	53.7	51	49	50	50	55.5	44.4	53.6	46.3	55.5	44.4	62.8	37.1
Piperacillin+Tazobactam	66.8	33.2	70.6	29.4	75	25	77.7	22.2	73.2	26.8	77.7	22.2	85.7	14.3

Where S=susceptible;R=resistant

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