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Original Research Article

Effect of addition of disodium EDTA to ceftriaxone and sulbactam on the susceptibility of gram-negative pathogens

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ABSTRACT

Background: Indiscriminate use of antibiotics has resulted in the emergence of resistant pathogens. Ethylenediaminetetraacetic acid (EDTA) is a well-known chelating agent. EDTA is also a potentiating and sensitizing agent when combined with antibiotics**.** Objectives of current study were to examine the antibacterial activity of Ceftriaxone+Sulbactam+ Disodium EDTA against pathogens isolated from urine, blood, and respiratory secretions (sputum, tracheal tube aspirates) and compare it with other antibiotics.
Methods: The study was a retrospective study conducted in a tertiary care hospital between January 2019 and December 2019. Gram-negative isolates were obtained from clinical samples of urine, blood and respiratory samples were tested for susceptibility to different antibiotics and also for ESBL and MBL production
Results: Respiratory isolates: Against *Acinetobacter baumannii*, 64% of isolates were sensitive to Ceftriaxone Sulbactam EDTA. Highest resistance rates were observed with Piperacillin tazobactam, amoxicillin clavulanate and Cefoperazone+Sulbactam. All Clinical isolates of *Pseudomonas* were sensitive to Ceftriaxone Sulbactam EDTA. Blood isolates: 25% isolates of Acinetobacter were sensitive to Ceftriaxone Sulbactam EDTA as compared to just 13% susceptibility for Cefoperazone+Sulbactam Urine isolates: 78% *E. coli* were sensitive to Ceftriaxone Sulbactam EDTA.
Conclusions: The combination of ceftriaxone, sulbactam and disodium edetate was effective even against pathogens isolated from isolates from respiratory secretions, blood and urine resistant to other antibiotics.

Key words: Ceftriaxone, Sulbactam and disodium edentate, *Acinetobacter baumannii*, ESBL

INTRODUCTION

Indiscriminate use of antibiotics has resulted in the emergence of resistant pathogens. Escalating resistance trends to antibiotics leads to an increase in hospitalizations, prolonged hospitalization and deaths due to infections. New drug development takes several years. Hence to deal with the current prevailing issues of antibiotic resistance the trend is to use antibiotic combinations, use beta lactamase inhibitors and antibiotic combinations. The synergistic effects of these antibiotic combinations may help eradicate the resistant pathogens.[1] Ethylenediaminetetraacetic acid (EDTA) is a well-known chelating agent. EDTA is also a potentiating and sensitizing agent when combined with antibiotics. The EDTA and antibiotic combination can be an innovative approach to deal with resistant pathogens. EDTA has a high affinity for metal ions and has a high density of ligands. EDTA binds to antibiotics through two amino and four carboxylate groups.[2] EDTA, itself does not have any significant antimicrobial activity. But, EDTA acts as a 'potentiator' of the activity of other antimicrobial agents.[3,4]

An antibiotic adjuvant entity (AAE) of ceftriaxone, sulbactam and disodium edetate as developed to address the challenge posed by multidrug resistant (MDR), extended spectrum beta lactamase (ESBL) producing pathogens or metallo-beta-lactamase (MBL) producing pathogens . A potent synergistic effect is expected to occur with this combination. Ceftriaxone is a cephalosporin while sulbactam is a beta-lactamase inhibitor and EDTA exerts its antibacterial action through antibiofilm and metal chelating properties. EDTA enhances the penetration of the antibiotics by increasing the membrane porosity and thus it causes a decrease in the minimum inhibitory concentration (MIC) of the antibiotics it is combined with.[5-7] The combination of ceftriaxone, sulbactam and disodium edetate has been approved by the Drug Controller General of India (DCGI) for the treatment of MDR, ESBL associated infections.[8]

Objectives

This retrospective in vitro study was conducted to examine the antibacterial activity of Ceftriaxone+Sulbactam+ Disodium EDTA against pathogens isolated from urine, blood and respiratory secretions (sputum, tracheal tube aspirates) and compare it with other antibiotics such as 3rd generation Cephalosporins (cefoperazone, ceftriaxone), Betalactam antibiotics and beta lactamase inhibitors (amoxycillin-clavulanic acid & Piperacillin-Tazobactam), Carbapenems (imepenem, meropenem) and tigecycline.

METHODS

The study was a retrospective study conducted in a tertiary care hospital (Yashoda Super Specialty Hospital) between January 2019 and December 2019.The inclusion criteria were gram negative isolates obtained from blood , urine, respiratory secretions samples collected from intensive care units (ICUs) patients and out patients for routine cultures. The exclusion criteria were isolates from samples other than blood, urine, respiratory secretions The sample size was calculated by using the estimated prevalence of resistance rates. This proportion was determined for the 95% percentile confidence intervals Isolates were obtained from urine (n=158), respiratory secretions (n=29) and blood (n=33).The gram-negative isolates were further screened for ESBL and MBL production. Screening of isolates for ESBL production was performed as per the Clinical Laboratory Standards Institute (CLSI) guidelines. Isolates exhibiting zone size ≤25 with ceftriaxone (30 µg), ≤22 for ceftazidime (30 µg), and ≤27 with cefotaxime (30 µg) were considered as possible ESBL producers. Extended-spectrum beta-lactamase production was confirmed by disk potentiation test using ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic disks with and without clavulanic acid (10 µg) and by double disk susceptibility test (DDST). Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion method as recommended by the CLSI (2020). The antibiotic susceptibility disks were purchased from from Hi-Media (Mumbai, India). Pathogens were isolated from specimens obtained from inpatients and out patients in a tertiary care hospital in Delhi NCR (India). Ethics committee approval was taken from the institutional ethics committee. The study was planned as a retrospective study of the data accruing from the microbiology department of the hospital. Antibiotic susceptibility was evaluated by the Kirby Bauer disc diffusion method and VITEK 2. Antibiotic susceptibility results were interpreted as per the CLSI guidelines (2020). The sample size was calculated to obtain a deference of 10% between the antibiotics studied and cefoperazone sulbactam EDTA with $p<0.05$ considered as significant.

RESULTS

Total 274 males and 227 females were included in the study. The mean age of the patients in the study was 56.84 years. In respiratory secretions the isolates included *Acinetobacter baumannii* (N=14), *Pseudomonas aeruginosa* (N=7), *Klebsiella pneumonia* (N=4), *Escherichia coli* (N=4). Against *Acinetobacter baumannii*, 64% isolates were sensitive to ceftriaxone sulbactam EDTA. Highest resistance rates were observed with Piperacillin tazobactam, amoxicillin clavulanate and Cefoperazone+Sulbactam. All Clinical isolates of *Pseudomonas* were sensitive to Ceftriaxone Sulbactam EDTA and only 65% isolates of *Pseudomonas* were sensitive to Tigecycline (Table 1). The chief isolates from blood included *Acinetobacter baumannii* (N=8), E coli (N=11), *Enterobacter* spp. (N=1), *Klebsiella pneumoniae* (N=11) and *Pseudomonas aeruginosa* (N=2). 25 % isolates of Acinetobacter were sensitive to Ceftriaxone Sulbactam EDTA as compared to just 13% susceptibility for Cefoperazone+Sulbactam. None of the other antibiotics were effective against *Acinetobacter baumannii* isolated from blood cultures. 18% isolates of *Klebsiella* were sensitive to Ceftriaxone Sulbactam EDTA while 50% isolates of Pseudomonas were intermediate sensitive to Ceftriaxone Sulbactam EDTA (Table 2). The chief isolates from urine included *Escherichia coli* (N=110), Klebsiella pneumoniae (N=19), *Pseudomonas* (N=17), *Proteus* (N=4), Citrobacter (N=3), *Acinetobacter baumannii* (N=2), Enterobacter (N=1) and *Morganella* (N=1). 78% *E. coli* were sensitive to Ceftriaxone Sulbactam EDTA (Table 3).

DISCUSSION

EDTA enhances antibiotic penetration by binding to the metal ions which compete with the antibiotics for the cell wall receptor. EDTA may also act by disruption of the lipopolysaccharides structure in the outer membrane of gram negative bacteria leading to increased permeability to the antibiotic.[1,4, 9-13] EDTA has a bacteriostatic activity against Gram-negative and Gram positive bacteria.[14] In the study by *Singh et al* ceftriaxone sulbactam EDTA (CSE) combination was the most effective antibiotic showing 94% sensitivity for carbapenem-sensitive Enterobacteriaceae and 97% for carbapenem-resistant *Acinetobacter* and *Pseudomonas* spp.[15]

Table 1: Comparison of antibiotic susceptibility trends for isolates from respiratory secretions.

BAL+Sputum+ ET secretion		Ceftriaxone Sulbactam EDTA		Cefoperazone + Sulbactam		Piperacillin tazobactam		Carbapenems		Tigecycline		Amoxycillin clavulanic acid	
		N	%	N	%	N	%	N	%	N	%	N	%
Acineto-bacter baumannii	S	9	64	0	0	0	0	0	0	0	0	0	0
	I	2	14	3	21	0	0	0	0	0	0	0	0
	R	3	21	11	79	14	100	14	100	11	100	2	100
E. coli	S	3	75	0	0	1	25	3	75	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	1	25	3	100	3	75	1	25	4	100	0	0
Klebsiella	S	1	25	1	33	2	50	2	50	1	25	0	0
	I	1	25	0	0	0	0	0	0	0	0	0	0
	R	2	50	2	67	2	50	2	50	3	75	0	0
Pseudo-monas	S	7	100	5	100	7	100	4	57	3	60	0	0
	I	0	0	0	0	0	0	1	14	0	0	0	0
	R	0	0	0	0	0	0	2	29	2	40	0	0
Total		29		25		29		29		24		2	

Table 2: Comparison of antibiotic susceptibility trends for isolates from blood.

Blood		Ceftriaxone Sulbactam EDTA		Cefoperazone + Sulbactam		Piperacillin tazobactam		Meropenem		Tigecycline		Amoxycillin clavulanic acid	
		N	%	N	%	N	%	N	%	N	%	N	%
Acinetobacter	S	2	25	1	13	0	0	0	0	0	0	0	0
	I	2	25	0	0	0	0	0	0	0	0	0	0
	R	4	50	7	88	8	100	8	100	4	100	0	0
E. coli	S	10	91	9	82	11	100	11	100	2	20	1	50
	I	0	0	0	0	0	0	0	0	1	10	0	0
	R	1	9	2	18	0	0	0	0	7	70	1	50
Enterobacter	S	1	100	1	100	1	100	1	100	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	1	100	0	0
Klebsiella	S	2	18	1	9	1	9	1	10	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	9	82	10	91	10	91	9	90	11	100	2	100
Pseudomonas	S	0	0	1	50	1	50	0	0	0	0	0	0
	I	1	50	0	0	0	0	0	0	0	0	0	0
	R	1	50	1	50	1	50	2	100	0	0	0	0
Total		33		33		33		32		26		4	

Patil et al studied the effects of CSE in 18 patients with septicaemia. 83.3% patients had complete clinical cure but 3 patients (16.6%) had treatment failure. 83.3% patients demonstrated a complete bacteriological eradication. No serious adverse effects were reported.[8] The current study has demonstrated superior activity of Ceftriaxone Sulbactam EDTA against isolates from respiratory secretions such as *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, as compared to Piperacillin tazobactam, amoxicillin clavulanate and Cefoperazone+Sulbactam. Lowest resistance rates were observed in the isolates from respiratory secretions for Ceftriaxone Sulbactam EDTA. In blood samples, 25 % isolates of *Acinetobacter* were sensitive to Ceftriaxone Sulbactam EDTA as compared to just 13% for Cefoperazone+Sulbactam . None of the other antibiotics were effective against *Acinetobacter baumanaii.* The chief isolates from urine included *Escherichia coli* (N=110), *Klebsiella pnseumonaie* (N=19), *Pseudomonas aeruginosa* (N=17), *Proteus mirabilis* (N=4), *Citrobacter spp* (N=3), *Acinetobacter baumanii* (N=2), *Enterobacter spp* (N=1) and *Morganella* (N=1).78% *Escherichia.coli* were sensitive to Ceftriaxone Sulbactam EDTA. The results of the current study indicate that the addition of EDTA improved the efficacy of Ceftriaxone Sulbactam EDTA. Pathogens resistant to other beta lactam antibiotics remained sensitive to Ceftriaxone Sulbactam EDTA.

Table 3: Comparison of antibiotic susceptibility trends for isolates from urine.

Urine		Ceftriaxone Sulbactam EDTA		Cefoperazone + Sulbactam		Piperacillin tazobactam		Meropenem		Tigecycline		Amoxycillin clavulanic acid	
		N	%	N	%	N	%	N	%	N	%	N	%
Acinetobacter	**S**	1	50	1	50	1	50	1	50	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	1	50	1	50	1	50	1	50	0	0	1	100
Citrobacter	**S**	3	100	3	100	3	100	2	67	1	50	1	100
	I	0	0	0	0	0	0	1	33	0	0	0	0
	R	0	0	0	0	0	0	0	0	1	50	0	0
E. coli	**S**	86	78	80	75	91	88	91	86	32	33	30	77
	I	3	3	3	3	0	0	3	3	7	7	1	3
	R	21	19	23	22	13	13	12	11	59	60	8	21
Enterobacter	**S**	1	100	1	100	1	100	1	100	0	0	1	100
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella	**S**	8	40	7	35	5	26	7	37	4	21	1	17
	I	1	5	1	5	1	5	2	11	2	11	0	0
	R	11	55	12	60	13	68	10	53	13	68	5	83
Morganella	**S**	0	0	1	100	1	100	1	100	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	R	1	100	0	0	0	0	0	0	1	100	0	0
Proteus	**S**	3	75	3	75	3	75	2	67	3	75	2	100
	I	0	0	0	0	0	0	1	33	0	0	0	0
	R	1	25	1	25	1	25	0	0	1	25	0	0
Pseudomonas	**S**	8	47	9	64	8	50	6	35	0	0	1	100
	I	0	0	5	36	0	0	1	6	0	0	0	0
	R	9	53	0	0	8	50	10	59	1	100	0	0
Total		158		151		150		152		125		51	

Limitations

Limitations of the study include the absence of CLSI guidance for new combination antibiotics like ceftriaxone sulbactam EDTA.A larger sample size will be required to corroborate the findings of this study. The study was a single center study and it cannot reflect the antibiotic susceptibility trends in the region.

CONCLUSION

The quest for new antibiotics continues in order to find solutions to the ever-increasing resistant pathogens. Until new antibiotics are developed combination of antibiotics has been considered to be the answer to the dilemmas of antibiotic resistance. The combination of ceftriaxone, sulbactam and disodium edetate has been found to be effective even against pathogens isolated from isolates from respiratory secretions, blood and urine resistant to other antibiotics. The formulation of the combination of Ceftriaxone, sulbactam and disodium edentate will be an important and affordable treatment option in the armamentarium of clinicians treating infections in the hospital and ICU setting.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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