COMPARATIVE SUSCEPTIBILITIES OF β - LACTAMASE PRODUCING ISOLATES OF PROTEUS MIRABILIS TO CEFTRIAXONE ALONE AND IN COMBINATION WITH SULBACTAM

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ABSTRACT

Objective: To evaluate the increase in Susceptibilities of Ceftriaxone resistant clinical isolates of Proteus mirabilis by combining sulbactam with ceftriaxone.

Material and Methods: An institution based analytic observational study was carried out at Department of Microbiology, Khyber Medical College Peshawar. b - lactamase producing Proteus mirabilis were identified and cultured in Mueller Hinton medium in Petri dishes. The antibiotics disks containing ceftriaxone alone and in combination with sulbactam were placed in the same Petri dishes about 24 – 30mm apart. The zones of inhibition were measured by following National Committee for Clinical Laboratory Standards zone diameter criteria in using disk diffusion method.

Results: The disks containing the combination of ceftriaxone and sulbactam showed larger zones of inhibition. The ceftriaxone alone had some anti bacterial activity against Proteus mirabilis. But by combining 20, 30 and 40 µg ceftriaxone with sulbactam in 1:1 ratio, the sensitivity was increased by 32.5%, 55% and 62.5% respectively. In 2:1 ratio, the combination produced 47.5%, 60% and 67.5% increase in sensitivity.

Conclusion: The sensitivities of Proteus mirabilis to Ceftriaxone can be restored by adding sulbactam.

Key Words: Proteus Mirabilis, Ceftriaxone, Sulbactam.

INTRODUCTION

β - lactam antibiotics have actually revolutionised the treatment of infections[1]. But with the over use of antibiotics bacterial resistance quickly became apparent[2]. Strong selective pressures of antimicrobial use in humans causes resistance[3]. Bacterial resistance against cephalosporins is most often mediated by β - lactamases[4]. β -lactamase inhibitors have negligible anti microbial activity themselves but restore antimicrobial activities of other -lactams when used in combination[5]. Use of the specific β - lactamase inhibitors in combination with a -lactam molecule is a logical attempt to overcome enzyme mediated resistance[6]. Sulbactam is one of the β - lactamase inhibitors. Its activity against extended spectrum β -lactamases is more uniform than other inhibitors[7]. Unlike clavulanic acid it does not induce production of β -lactamases[8]. Also sulbactam combination have favourable safety profiles and are well tolerated.[9,10] Sulbactam combines with some clinically relevant β - lactamases in an irreversible manner. If sufficient inhibitor is present at the site of infection, β - lactamase enzymes should be neutralized and thus the drug should have an opportunity to inhibit bacterial growth.[11] The objective of the present study was to see whether or not combination of sulbactam with ceftriaxone potentiates, the antibacterial activity against Proteus mirabilis.

MATERIAL AND METHODS

Proteus mirabilis isolated from the clinical laboratory specimens were cultured and identified by their morphology, cultural characteristics and biochemical activity. Out of these isolates 40 - lactamase producing strains were isolated by a

rapid chromogenic method, using chromogenic cephalosporin sticks[12]. The impregnated end of the stick was moistened and touched with a bacterial colony, picking up a small cluster of cells.

The tip was examined for up to five minutes. The development of pink/ red colour indicated a positive reaction.[13] For preparation of media, Mueller Hinton agar[14] was used. It has now become the standard for Baur-Kirby method and its performance is specified by National Committee of Clinical Laboratory Standards (NCCLS)[15] The Proteus mirabilis that had been subcultured on nutrient agar and re-identified, were inoculated in 2ml of Mueller Hinton broth in Beiju bottles and incubated at 37°C aerobically for 18 hours. Then turbidity of the suspension was adjusted at 10^5 – 10^6 CFUs/ ml[16] with sterile Mueller Hinton broth and checked against Mc Farlands turbidity standard barium sulfate[17]. Then broth was poured over the standard Mueller Hinton agar plates.

For preparation of the antibiotic disks, 6mm filter paper chips were sterilized at 160°C in a hot air oven and after allowing the disks to cool, 20µl of the required concentration of Ceftriaxone solution was poured on each disk[18].

Disks of 20, 30 and 40 µg concentration of ceftriaxone alone and with sulbactam were prepared and placed on Mueller Hinton culture plates with about 24mm distance between the consecutive disks. The plates were incubated at 35 – 37°C for about 16 – 18 hours. The diameter of zone of inhibition around each disk was measured which corresponded to the activity of each disk. Zone diameter of 13 – 21 mm was taken as sensitive.

RESULTS

The susceptibilities of all 40 β -lactamase producing isolates were recorded by using antibiotic disks of 20, 30 and 40 µg ceftriaxone and the disks containing same concentration of this antibiotic with β - lactamase inhibitor sulbactam in 1:1 and 2:1 ratios. The ceftriaxone alone had some anti bacterial activity against Proteus mirabilis. But by combining 20, 30 and 40 µg ceftriaxone with sulbactam in 1:1 ratio, the sensitivity was increased by 32.5%, 55% and 62.5% respectively. In 2:1 ratio, the combination produced 47.5%, 60% and 67.5% increase in sensitivity. The results obtained by measuring the zones of inhibition of different disks are shown in Table1 and fig1.

DISCUSSION

The novel therapy of combining an established β-lactam antibiotic with β-lactamase inhibitor neutralizes the effect of β- lactamases[19]. Persuing the same principle the present study has been conducted.

The global emergence and spread of antimicrobial resistance poses a major risk for human health due to the impact on morbidity mortality, and health care costs[20]. The Study envisages to meet the same challenge.

Table 1 and fig 1 delineates the sensitivity of Proteus mirabilis against cefriaxone alone and in combination with sulbactam. The ceftriaxone alone has some anti bacterial activity against Proteus mirabilis. But by combining 20, 30 and 40 µg ceftriaxone with sulbactam in 1:1 ratio, the sensitivity is increased by 35%, 80% and 85% respectively.

In 2:1 ratio, the combination produced 65%, 90% and 87.5% increase in sensitivity. This shows that the combination of ceftriaxone with sulbactam has significant synergistic effect to restore the sensitivity of resistant strains of Proteus mirabilis. This effect is specially pronounced in 1:1 ratio, which means in a ratio with sufficient Sulbactam concentration.

Sulbactam combined with ampicillin, mezlocillin and cefoperazone is currently used in medical practice and separate agents are also being tested in preliminary clinical trails[22,23]. In certain countries sulbactam is available as a single agent for co-administration with antibiotics as the

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Ceftriaxone (Control)		Ceftriaxone + Sulbactom (1:1)			Ceftriaxone + Sulbactom (2:1)		
Cone (µg)	Sensitive	Sensitive	Increase in sensitivity	% increase in sensitivity	Sensitive	Increase in sensitivity	% increase in sensitivity
20	1	14	13	32.5	20	19	47.5
30	2	24	22	55	26	24	60
40	4	29	25	62.5	31	27	67.5

Table 1

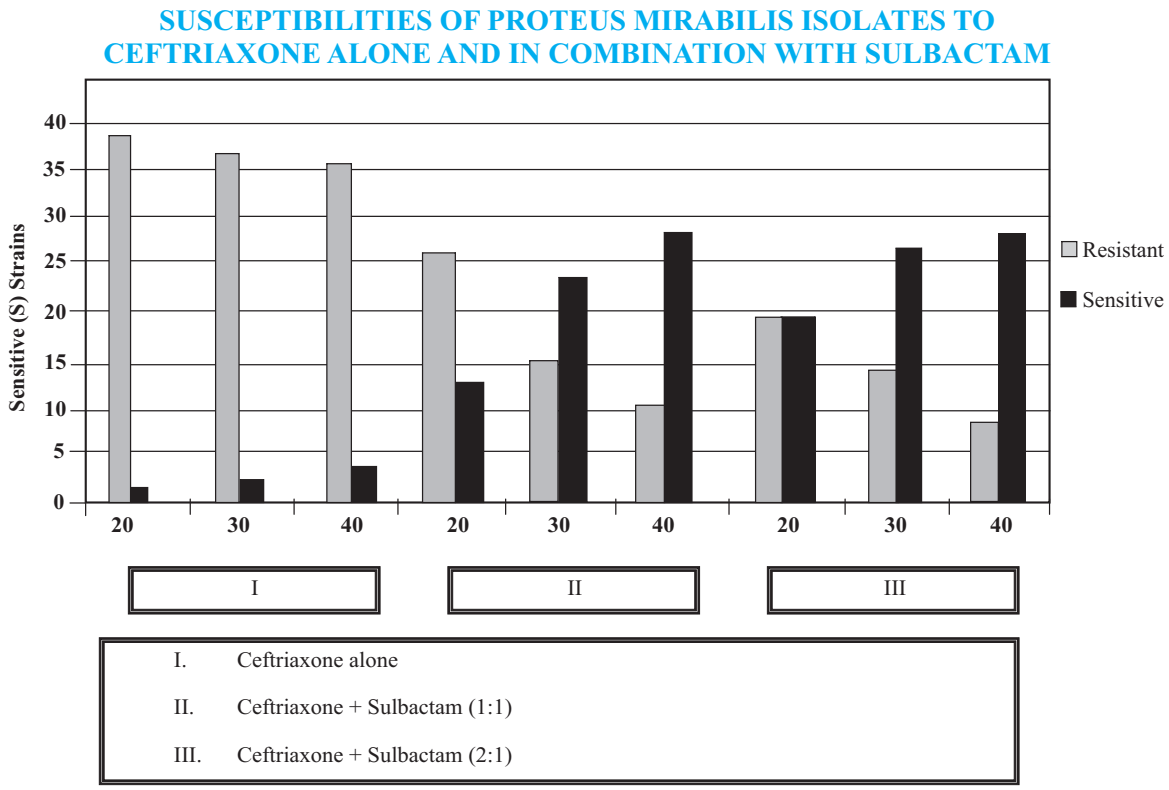

Fig.1

prescriber sees fit[23].

Research workers have also found that the full potential of cefoperazone against the enterobacteriaceae was restored by the addition of sulbactam[22]. In some studies cross resistance was observed with all third generation cephalosporins[24]. These studies provide ample justification for our work on a third generation cephalosporin, ceftriaxone which is unique regarding its excretion which is through the kidneys as well as in the bile[25].

Proteus mirabilis being the member of enterobacteriaceae were logically chosen for this highly needed synergism. Our data also provides us guidelines to solve the problem of wide spread resistance to third generation cephalosporin antibiotics. As the indiscriminate use of higher generation cephalosporins has deprived these drugs of their maximum benefit in many cases when these are really indicated. Hectic efforts are needed to study and formulate the combinations of higher generation cephalosporins, with the older as well as the newer β - lactamase inhibitors in the hope to restore the lost antimicrobial activity of these β - lactams[1]

CONCLUSION

The susceptibilities of Proteus mirabilis can be restored by combining sulbactam with ceftriaxone.

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