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In vitro Antimicrobial Activity of Cephradine and Beta-lactamase Inhibitors, Alone and in Combination Against Clinical Bacterial Isolates of *Escherichia coli*

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SUMMARY

Beta-lactamases present the greatest single challenge to beta-lactam antibiotics. In the present study 135 clinical isolates of Escherichia coli were observed for beta-lactamase production and 40 of them were found to be beta-lactamase producing. These beta-lactamase producing isolates were tested for their susceptibility to cephradine alone and then against the cephradine-clavulanic acid and cephradine-sublactam in three different concentrations and two different ratios by disk diffusion method. The results were interpretted according to NCCLS (1995) zone diameter criteria. Cephradine alone showed insignificant antibacterial activity. The combination of cephradine-clavulanic acid showed upto 57.5 percent while the cephradine-sulbactam combination showed upto 70.0 percent increase in sensitivity against Escherichia coli.

INTRODUCTION

Bacterial resistance to the beta-lactam drugs is extremely widespread as a result of extensive use. Loss of susceptibility is primarily attributable to hydrolysis of beta-lactam ring by inactivating enzymes, namely the beta-lactamases[1,2]. These beta-lactamases may either be chromosomally or plasmid-mediated[3]. Several effective strategies have been implemented in order to overcome the beta-lactamase-mediated resistance. One approach has been through the development of beta-lactamase inhibitors. The beta-lactamase inhibitors include clavulanic acid[4], sulbactam and tazobactam[5]. When these inhibitors are combined with safe and efficacious penicillins or cephalosporins, they can serve to protect the familiar beta-lactam antibiotics from hydrolysis. These beta-lactamase inhibitors eventually inactivate the target enzymes permanently and are referred to as suicidal inhibitors[6].

While using beta lactam antibiotics and beta-lactamase inhibitor combinations, synergism has been observed in a number of studies. The combinations of clavulanic acid with amoxycillin[7], sulbactam with ampicillin[8] and sulbactam with cefoperazone[9] have successfully increased the antibacterial spectrum of these beta-lactam antibiotics. The increased synergy has been demonstrated for most members of the Enterobacteriaceae, Neisseria gonorrhoeae, Hemophillus influenzae and Penicillinase producing Staphylococcus aureus. These antibiotic combinations have been used clinically to treat urinary tract infections, bone and soft tissue infections, gonorrhea, respiratory infections and otitis media.

The objective of the present study was to compare the in-vitro antibacterial activities of cephradine alone and in combination with beta-lactamase inhibitors (clavulanic acid and sulbactam) against clinical isolates of Escherchia coli.

MATERIALS AND METHODS

The micro-organisms included in this study were selected from amongst isolates grown from clinical specimens in the Microbiology Laboratory of Khyber Medical College. The drugs used were cephradine (Squibb Pharmaceuticals) clavulanic acid (Beecham Research Laboratories) and sulbactam (Pfizer Pakistan Ltd.). The organisms that appeared resistant to cephradine were tested for beta-lactamase production by chromogenic beta-lactamase method[10], using oxoid beta-lactamase identification sticks (Code: BR 66). The beta-lactamase positive organisms were then preserved on nutrient agar slopes for susceptbility testing. The organisms to be tested were then subcultured on blood agar, nutrient agar, and MacConkey agar and incubated at 37°C aerobically overnight. These organisms were re-identified by screening methods before subjecting them to disk diffusion studies. The sensitivity tests were performed by NCCLS disk test procedure[11] which is a modified form of Baur-Kirby method[12]. Muller-Hinton[13] agar (oxoid) was used for culture and sensitivity testing of the microorganisms. The antimicrobial disks containing three different concentrations of cephradine alone (20, 30 & 40 μg) and in combination with clavulanic acid/sulbactam in two different ratios (1:1 and 2:1) were used for sensitivity testing. In order to control the precision of the test procedure positive and negative controls were used. Escherichia coli. ATCC 25922 was used as positive control and uninnoculated medium as negative control.

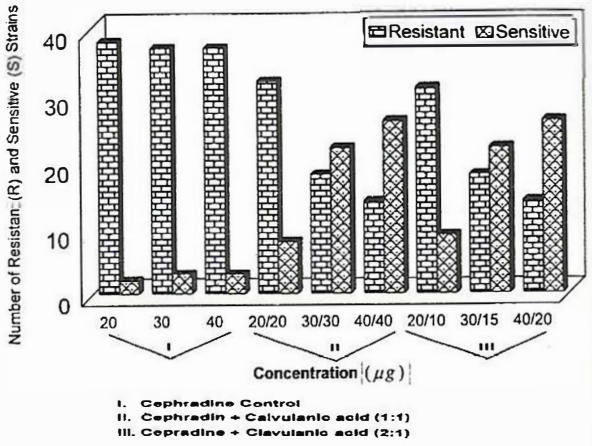

Fig. 1: Effect of clavulanic acd on the antibacterial activity of cephradine against Escherichia coli.

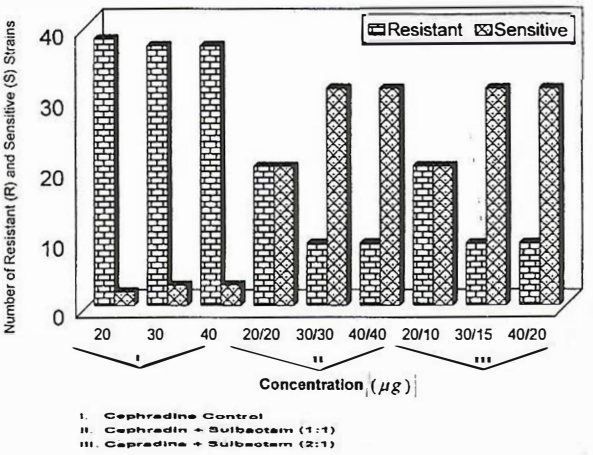

Fig. 2: Effect of sulbactam on the antibacterial activity of cephradine against Escherichia coli.

RESULTS

The results were interpreted as Sensitive(S) and Resistant(R) according to National Committee for Clinical Laboratory Standards (NCCLS., 1995) zone diameter criteria and are shown in Tables 1, 2 and Figs. 1, 2. The current NCCLS criteria defines zone size breakpoint for Escherichia coli as being Resistant (Zone dia < 16 mm) and Sensitive (Zone dia. > 18 mm). Of the 40 isolates only 3 (7.5%) were sensitive while the rest i.e. 37 (92.5%) were resistant when tested with cephradine alone against the maximum concentrations (i.e. 30 μg and 40 μg). This showed that the antibacterial activity of cephradine was insignificant. When cephradine was combined with clavulanic acid in both the ratios (1:1 and 2:1), the number of sensitive isolates increased with increasing concentrations and a maximum number of 26 (65.0%) was observed with 40/40 μg and 40/20 μg concentrations. With the addition of sulbactam in both the ratios, the number of sensitive isolates increased to 31 (77.5%) when tested against the maximum concentrations (30/30, 40/40, 30/15, 40/20 μg), showing highly significant antibacterial activity.

Table 1: Effect of clavulanic acid on the antibacterial activity of cephradine against Escherichia coli (n=40).

Cephradine (Control)			*Cephradine + Clavulanic acid (1:1)*				*Cephradine + Clavulanic acid (2:1)*			
Conc.(µg)	*Resistant*	*Sensitive*	*Resistant*	*Sensitive*	*X2*	*P.Value*	*Resistant*	*Sensitive*	*X2*	*P.Value*
20	38	2	32	8	2.8	>0.05	31	9	3.7	>0.05
30	37	3	18	22	18.8	<0.001	18	22	18.8	<0.001
40	37	3	14	26	26.1	<0.001	14	26	26.1	<0.001

Table 2: Effect of sulbactam on the antibacterial activity of cephradine against Escherichia coli (n=40).

Cephradine (Control)			*Cephradine + Sulbactam (1:1)*				*Cephradine + Sulbactam (2:1)*			
Conc.(µg)	*Resistant*	*Sensitive*	*Resistant*	*Sensitive*	*X2*	*P.Value*	*Resistant*	*Sensitive*	*X2*	*P.Value*
20	38	2	20	20	18.1	<0.001	20	20	18.1	<0.001
30	37	3	9	31	37.2	<0.001	9	31	37.2	<0.001
40	37	3	9	31	37.2	<0.001	9	31	37.2	<0.001

n = the number of beta-lactamase producing isolates.
X^2 is the Chi square.
P. value of Cephradine (Control) and the combinations.

DISCUSSION

The spectrum of antibacterial activity of beta-lactam antibiotics (penicillins and cephalosporins) is limited due to their susceptibility to beta-lactamase enzymes produced by commonly encountered gram-positive and especially gram-negative pathogens. The use of beta-lactamase inhibitors in combination with some beta-lactam antibiotics is one of the approaches to counteract the resistance due to beta-lactamases. These are irreversible inactivators of beta-lactamases. When these inhibitors are present in sufficient concentrations at the site of infections, the beta-lactamases are neutralized and the drug used in combination with the inhibitor has an opportunity to inhibit bacterial growth.

This study showed that cephradine alone has very little antibacterial activity against Escherichia coli in all the three concentrations. The combination of cephradine with clavulanic acid showed 15.0, 47.5 and 57.5 percent increase in the sensitivity of this micro-organism with three different concentrations respectively. With cephradine-sulbactam combination in both the ratios there was 45.0, 70.0 and 70.0 percent increases in the sensitivity of this organism with three different concentrations respectively. It was further observed that cephradine-sulbactam produced better synergism as compared to cephradine-clavulanic acid in both the ratios which may be due to increased bacterial outer membrane permeability of Sulbactam[14,15]. It is also evident that these combinations of cephradine and clavulanic acid/sulbactam in both ratios produced similar results. Our these findings are similar to the observations made by other authors[16].

This data suggests that beta-lactamase inhibitors (clavulanic acid/sulbactam) are very useful compounds which can re-expand the spectrum of first generation cephalosporins against beta-lactamase producing Escherichia coli encountered in out-patient or indoor-patients.

A detailed in-vivo investigations regarding the synergistic activity of beta-lactamase inhibitors will help in developing suitable means for revival of the older and safer drugs to which most of the organisms have developed resistance.

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