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The Interaction Between Gentamicin and Floroquinolones Against ESBL Producing Clinical Isolates of *Escherichia coli*

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Abstract: The present study evaluated the antimicrobial effect of different combinations of gentamicin and floroquinolones (ciprofloxacin, ofloxacin, perfloxacin and gatifloxacin) against extended spectrum beta lactamase (ESBL) positive *E. coli* isolates using checkerboard method. One hundred and four clinical isolates of *E. coli* obtained from urine (26), blood (24), stool (20), sputum (19) and semen (14) were investigated for ESBL production. Fifteen (14.4%) were positive for ESBL production in the following order: 4(3.8%) from urine, 3(2.8%) from blood, 3(2.8%) from stool, 3(2.8%) from sputum and 2(1.9%) from semen. Five representative ESBL positive isolates, one from each specimen, were investigated for their susceptibility patterns to different gentamicin and floroquinolone combinations. The combinations of gentamicin and ciprofloxacin, perfloxacin and gatifloxacin, respectively at different ratios were predominantly synergistic in activity while gentamicin and ofloxacin combinations were primarily indifference in activity. These results may have some therapeutic significance in the management of ESBL infections especially in areas of the world where ESBL organisms are either emerging or re-emerging.

Key words: Combination, antimicrobial, isolate, ESBL enzyme, susceptibility, therapeutic

INTRODUCTION

Extended Spectrum Beta-Lactamase (ESBL) producing strains of Enterobacteriaceae have emerged as a major problem in hospitalized as well as community based patients (Chaudhury and Aggarwal, 2004; Rodriquez-Bano *et al*., 2004). The organisms are implicated in infections such as Urinary Tract Infections (UTI), septicemia, hospital-acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections. The emergence and spread of ESBL-producing Enterobacteriaceae in intensive care units (ICU) is said to be due to clonal dissemination of a few epidemic strains as well as horizontal transmission of resistance gene-carrying plasmids among bacterial organisms (Wu *et al*., 2003). The extensive use of oxyimino-cephalosporins in medical institutions has resulted in diminished susceptibility of some enterobacteriaceae. This resistance has spread to strains of *E. coli* and other gram-negative bacteria (Iroha *et al*., 2008). The resistance is probably because of the presence of extended-spectrum beta-lactamase (ESBL) enzyme that was derived from the wide spread TEM-1/2 and SHV-1 family.

Aibinu *et al*. (2003) suggested the use of aminoglycosides, floroquinolones and cabapenems in preference to cephalosporins in the treatment of ESBL infections. Investigations have shown that ESBL enzymes also confer resistance to other classes of antibiotics (Fashae *et al*., 2004). Recent studies have shown however, that ESBL producing organisms have started developing resistance to the aminoglycosides and floroquinolones, thus posing serious therapeutic consequences. This study

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was therefore designed to investigate the *in vitro* therapeutic outcome of combining an aminoglycoside (gentamicin) and some floroquinolones including ciprofloxacin, ofloxacin, perfloxacin and gatifloxacin at different ratios against *E. coli* isolates expressing ESBL enzymes.

MATERIALS AND METHODS

Sample Collection

One hundred and four clinical isolates of *E. coli* were collected from the intensive care unit of Eastern Nigeria Medical Centre Enugu. The isolates were obtained from urine (26), blood (24), stool (20), sputum (19) and serum (14). They were subsequently identified and characterized using standard microbiology techniques (Chessbrough, 2002).

Culture Media

Mueller-Hinton Agar (Oxoid UK) and Nutrient Broth (Fluka) were prepared according to manufacturer's specifications.

Susceptibility Studies

This was conducted using the disc diffusion method as described by Bauer *et al*. (1966). Antibiotics tested were gentamicin (25 μg), nalidixic acid (25 μg), ceporex (30 μg), ofloxacin (25 μg), cipofloxacin (25 μg), perfloxacin (25 μg), ampicillin (25 μg), septrin (25 μg), amikacin (25 μg), ceftazidime (30 μg), cefotazime (30 μg), ceftriaxone (30 μg) and imipenem (30 μg).

Double Disc Synergy Test

Test isolates showing resistance to any of the 2nd and 3rd generation cephalosporins were subjected to double disc synergy test studies. Individual discs containing 30 μg of ceftazidime, ceftriaxone and cefotaxime were placed on the plate at a distance of 15 mm (edge to edge) from an amoxicillin/clavulanic acid disc (20-10 μg) placed at the center of the plate. An enhanced zone of inhibition between any of the β-lactamase discs and the disc containing clavulanic acid was interpreted as a positive result.

Preparation of Drug Stock Solution

A stock solution of gentamicin was prepared by dissolving in appropriate quantity of sterile distilled water to get 10,000 μg mL^{-1} and stock solutions of perfloxacin, ciprofloxacin, ofloxacin and gatifloxacin were dissolved in appropriated quantity of sterile distilled water to get 5,000 μg mL^{-1}. Seven different 2-fold arithmetical serial dilutions were made with each drug at their stock concentration.

Interaction Studies

Stock solutions of gentamicin (10,000 μg mL^{-1}), ciprofloxacin, ofloxacin, gatifloxacin and perfloxacin (5,000 μg mL^{-1}) were freshly prepared for the evaluation of their combined effects against five representative ESBL positive *E. coli* isolate from each specimen. The two agents were mixed in varying ratios ranging from 0:10 gentamicin to ciprofloxacin, perfloxacin, ofloxacin and gatifloxacin and 10:0 of same agents in accordance with the continuous variation checkerboard protocol (Esimone *et al*., 1999; Okore, 1990). Each of the eleven combinations of these five agents was serially diluted (2-fold) with sterile distilled water. One milliliter of each of the drug combinations was respectively seeded in a Petri dish together with 19 mL of sterile Mueller Hinton agar and allowed to stand for 1 h to solidify and for pre-diffusion of the drugs. An aliquot equivalent to 0.5 Macfarland standard of each selected ESBL producing *E. coli* was streaked on the surface of the agar plates. The set up was conducted in

triplicates with a control containing no antibiotics. They were then incubated at 37°C for 24 h. The MICs of the various combination proportions were determined and interactions between the antimicrobial agents were accessed by determining their fractional inhibitory concentrations (FIC) index according to the relationship.

FIC index = FIC_A+FIC_B where A and B are two antimicrobial agents being combined.
FIC_A = (MIC of drug A in combination with drug B)/(MIC of drug A alone)
FIC_B = (MIC of drug B in combination with drug A)/(MIC of drug B alone)
The activity index (AI) = Log FIC index.

RESULTS AND DISCUSSION

The result of the study revealed that 15 (14.42%) out of 104 clinical isolates of *E. coli* screened were positive for ESBL enzyme production. The highest frequency occurred in urine specimen 4 (3.8%) while the least occurred in semen 2(1.9%). The ESBL enzyme present is predominantly of SHV and TEM type. This was inferred from the fact that ceftazidime was the antibiotic that showed increase in the zone of inhibition above 5 mm towards the combination disc (amoxicillin/clavulanic acid).

The combination interaction studies showed that some ratios of antibiotic combinations were synergistic while some were additive, antagonistic and indifference in activity, respectively. The combinations of gentamicin and gatifloxacin, ciprofloxacin and perfloxacin were predominantly synergistic (Table 1-3), while that with ofloxacin were mainly indifference (Table 4). The combination ratio 8:2 produced the highest synergy (80%) for gatifloxacin combination and 3:7 and 9:1 for

Table 1: Activity of gentamicin and gatifloxacin against five ESBL producers by checkerboard method

Drug combination A: B	Average activity index for five ESBL producers	Synergism (%)	Antagonism (%)	Indifference (%)	Additivity (%)
0:10	-	-	-	-	-
1:9	-0.302	60	20	20	-
2:8	0.06	20	40	40	-
3:7	-0.01	60	20	20	-
4:6	-0.15	60	20	20	-
5:5	0.054	40	20	20	20
6:4	-0.054	60	20	20	-
7:3	-0.046	40	20	40	-
8:2	-0.246	80	20	-	-
9:1	-0.176	60	20	20	-
10:0	-	-	-	-	-

Table 2: Combined activity of gentamicin ant ciprofloxacin against five ESBL producers by checkerboard method

Drug combination A:B	Average activity index for five ESBL producers	Synergism (%)	Antagonism (%)	Indifference (%)	Additivity (%)
0:10	-	-	-	-	-
1:9	-0.226	60	-	40	-
2:8	-0.252	60	20	20	-
3:7	-0.094	80	-	20	-
4:6	0.132	40	40	-	20
5:5	0.11	-	20	60	20
6:4	-0.016	60	20	20	-
7:3	-0.156	60	-	40	-
8:2	-0.186	60	-	40	-
9:1	-0.292	80	-	-	20
10:0	-	-	-	-	-

Table 3: Combined activity of gentamicin and perfloxacin against five ESBL producers by checkerboard method

Drug combination A:B	Average activity index for five ESBL producers	Synergism (%)	Antagonism (%)	Indifference (%)	Additivity (%)
0:10	-	-	-	-	-
1:9	-0.486	100	-	-	-
2:8	-0.374	80	-	20	-
3:7	-0.336	60	20	20	-
4:6	-0.218	80	20	-	-
5:5	-0.236	60	20	20	-
6:4	-0.446	100	-	-	-
7:3	-0.414	80	-	20	-
8:2	-0.206	60	20	20	-
9:1	-0.446	80	-	20	-
10:0	-	-	-	-	-

Table 4: Combined activity of Gentamicin ant ofloxacin against five ESBL producers by checkerboard method

Drug combination A:B	Average activity index for five ESBL producers	Synergism (%)	Antagonism (%)	Indifference (%)	Additivity (%)
0:10	-	-	-	-	-
1:9	-0.220	40	-	60	-
2:8	0.152	-	-	100	-
3:7	-0.092	60	-	40	-
4:6	-0.094	60	20	20	-
5:5	-0.052	40	20	40	-
6:4	0.170	40	40	20	-
7:3	-0.098	40	20	40	-
8:2	-0.122	20	-	60	-
9:1	0.112	60	20	20	-
10:0	-	-	-	-	-

ciprofloxacin combination. A (100%) synergy was recorded for perfloxacin combinations at the ratio of 1:9 and 6:4. However, the highest indifference activity of ofloxacin combination was obtained at 2:8 ratio.

ESBL producing organisms are known to be resistant to beta lactam antibiotics. This is because ESBL producers have enzymes with relaxed active site that can encompass the beta lactam with large side groups protecting the beta lactam ring, thus making these organisms resistant to virtually all beta lactam antibiotics. This enzyme have spread world wide with intra and inter-species transfer being facilitated by plasmid encoded enzymes. The present study indicated 14.4% prevalence of ESBL producing *Escherichia coli* in Enugu, Nigeria. This figure is slightly less than the 16.5% prevalence reported from Abakaliki, Nigeria (Iroha *et al*., 2008). The recovery of ESBL producing *E. coli* in Enugu, Nigeria could pose a lot of clinical challenges especially in recent times when antibiotics other than the beta lactams are reported to develop resistance (Shannon and French, 2004). This later development is worrisome, since the aminoglycosides , floroquinolones and cabapenems were hitherto recommended as alternative to cephalosporins for ESBL producing organisms (Aibinu *et al*., 2003). In this connection, the need to search for effective substitute remedy against ESBL producers remains a major challenge to scientists.

The result of the interaction study in the present study indicated the predominance of synergy in the combinations of gentamicin and ciprofloxacin, perfloxacin and gatifloxacin. Hundred percent synergy was recorded for the gentamicin/perfloxacin combination at the ratios of 1:9 and 6:4 (Table 4). This discovery is very gratifying and brightens the hope for the remedy search for ESBL enzyme producers.

The synergistic interaction between gentamicin and ciprofloxacin recorded in this study is in line with the report of Mandal *et al.* (2003), in which combination of the two antibiotics produced

enhanced activity against ESBL producing enteric organism. Further, a study carried out in the United States established that gatifloxacin was synergistic with beta lactams including piperacillin, cefepime and meropenem and with gentamicin, against some drug resistant pathogens (Dawis *et al*., 2003). It is undoubtable from the result of the present study, that drug combination could be the longed-for panacea needed for the management of ESBL producing strains of *E. coli* and perhaps other organisms.

A further study on the drug interaction against other ESBL producing organisms is hereby emphasized. In conclusion, the enhanced *in vitro* activity resulting from antibiotic combinations in this study could be of immense significance in the successful treatment of most fatal bacterial diseases. This finding is particularly vital in the developing nations, where single effective drugs against ESBL producing organisms are either expensive or unavailable. Consequently, the establishment of such drug combinations for all the common ESBL producing microorganisms is at present, a compelling necessity, if a successful battle against ESBL producers will be achieved.

REFERENCES

Aibinu, I., P. Odugbemi and J.M. Brian, 2003. Extended-spectrum β-lactamase in isolates of *Klebsiella* sp. and *Escherichia coli* from Lagos. Nig. J. Health Biomed. Sci., 2: 53-60.

Bauer, A.M., W.M.M. Kirby, J.C. Sherris and M. Turk, 1966. Antibiotic susceptibility testing using a standard single disc method. Am. J. Clin. Pathol., 45: 493-496.

Chaudhury, U. and R. Aggarwal, 2004. Extended spectrum β-lactam (ESBL)-An emerging threat to clinical therapeutics. Indian J. Med. Microbiol., 22: 75-80.

Chessbrough, M., 2002. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, Cambridge, UK., ISBN 0-521-66546-9,.

Dawis, M.A., H.D. Isenberg., K.A. France and S.G. Jenkins, 2003. *In vitro* activity of Gatifloxacin alone and in combination with cefepime, meropenem, piperacillin and gentamicin against multi-resistant organisms. J. Antimirob. Chemother., 51: 1203-1211.

Esimone, C.O., M.U. Adikwu and O.P. Udeogaranya, 1999. The effect of ethylene diamine tetra acetic acid on the antimicrobial properties of benzoic acid and Cetrimide. J. Pharmaceut. Res. Drug Dev., 4: 1-8.

Fashae, K., I. Aibinu, F. Ogunsola, T. Odugbemi and B.J. Mee, 2004. Extended spectrum beta lactamase (ESBL) in *Klebsiella pneumoniae* isolates from septicaemic children in Ibadan, Nigeria. Nig. J. Health Biomed. Sci., 3: 79-84.

Iroha, I.R., M.U. Adikwu., E.S. Amadi., I. Aibinu and C.O. Esimone, 2008. Characterization of ESBL producing *E. coli* from secondary and tertiary hospital in south eastern Nigeria. Res. J Microbol., 3: 514-519.

Mandal, S., M.D. Mandal and N.K. Pal, 2003. Combination effect of ciprofloxacin and gentamicin against clinical isolates of Salmonella enteric serovar typhi with reduced susceptibility to ciprofloxacin. Jap. J. Infect. Dis., 56: 156-157.

Okore, V.C., 1990. Combination chemotherapy: *In-vivo* synergy between ampicillin and tetracycline against susceptible and resistant isolates of *Staphylococcus aureus*. W. Afi. J. Biol. Applied Chem., 35: 1-4.

Rodriquez-Bano, J., M.D. Navarro, L. Romero, L. Martinez-Martinez, M.A. Muniain and E.J. Perea, 2004. Epidermiology and clinical features of infections Caused by extended spectrum beta-lactamase producing *Escherishia coli* in non Hospitalized patients. J. Clin. Microbiol., 42: 1089-1094.

Shannon, K.P. and G.L. French, 2004. Increasing resistance to antimicrobial agents of Gram-negative organisms isolated at a London teaching hospital from 1995-2000. J. Antimicrob. Chemother., 53: 818-825.

Wu, T.L., J.H. Chia, L.H. Su, A.J. Kuo, C. Chu and C.H. Chiu, 2003. Dissemination of extended-spectrum ß-lactamase producing Enterobacteriaceae in pediatric intensive care units. J. Clin. Microbiol., 41: 4836-4838.