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Full Length Research Paper

Antimicrobial resistance pattern of plasmid-mediated extended-spectrum β-lactamase producing strains of *Escherichia coli*

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One hundred and twenty three clinical isolates of *Escherichia coli* isolated from Eastern Medical Hospital Enugu from blood (79) and urine (44) were bacteriologically analyzed for extended spectrum β-lactamase enzyme (ESBL) expression using double disc synergy testing method. Confirmed ESBL producing isolates were screened with nineteen different conventional antibiotics from different classes using reference agar diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS) to determine their resistance patterns. ESBL production were detected in 21 (17.0%) of the clinical isolates of *E. coli* from blood while 10 (8.1%) where from blood samples. In all 31 (25.2%) of clinical isolates from blood and urine produces ESBL phenotypically. The resistance profile studies showed that the ESBL producing organisms were multi-drug resistant and the highest resistance was observed with tetracycline, erythromycin, ampicillins, cephalosporins nitrofurantoin and floroquinolones while gatifloxacin have a tremendous activity against ESBL isolates followed by augmentin.

Key words: ESBL, clinical isolates, antimicrobial resistance.

INTRODUCTION

The present increase in resistance to second and third general cephalosporins observed in our medical institutions as a result of the acquisition and expression of extended-spectrum β-lactamase enzymes among Enterobactericeae has posed a serious public health problem. The clinical implications are extremely serious and lack of sensitive diagnostic method needed to guide therapy, monitor resistance developments and implementing intervention strategies have complicated the problem (Bradford, 2001; Sturenburg et al., 2003). ESBL producing strains have variable susceptibility rates for floroquinolnes, aminoglycosides and fourth generation cephalorsporins (Lautenbach et al., 2001). The carbapenems are the only class of antibiotics commonly active against ESBL although, ESBLs are known to be multi-drug resistance.

ESBLs are derivatives of simple β-lactamase (TEM or SHV) enzymes that are harboured mostly by Gram-negative bacilli. Selective pressure by the used of 2nd and 3rd generation cephalosporins favours the development of mutations that results in conformational changes in the active serine site of amino acid sequence of TEM or SHV enzymes (Szabo et al., 1997). As a result of these mutational changes these organisms have acquired an extra gene copy that makes them to develop resistance to a wide range of antibiotics which they were previously susceptible. Studies have shown that treating ESBL with cephalosporins always give poor therapeutic outcome and an alternative choice of antibiotics are the quinolones and aminoglycosides. The choice of investigating ESBL production from *E. coli* in Eastern part of Nigeria and its resistance patterns in the present study is because of the havoc ESBL producing organism is causing in most hospital in Western part of Nigeria. We therefore, decide to (a) investigate the presence and prevalence of ESBL in Eastern part of Nigeria, (b) the resistance patterns of ESBL if present to a wide range of antibiotics in our community because of the difficulties in selecting appropriate antibiotic for therapy against ESBL infections, as they are multi-drug resistant. We hope that our help in selecting

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antibiotics that could serve as an alternative in treating ESBL infection.

METHODS

Bacteria isolates

One hundred and twenty three clinical isolates of *E. coli* were isolated from blood (79) and urine (44) from Eastern Medical Hospital Enugu, Nigeria. These clinical isolates were collected from patients in the intensive care unit and pediatric wards suffering from peritonitis, neurosurgical meningitis and children with skin and soft tissue infection (Septicemia). Unfortunately two children died as a result of septicemia infection, and the specimen we collected from one of them was positive for ESBL. The samples were identified and characterized using standard microbiological techniques (Cowan and Stell, 1983).

Antimicrobial susceptibility studies

Antibiotic testing

Molten Mueller-Hinton agar plates were prepared and the test organisms inoculated with a sterile swab stick, which were incubated at 37^0C for I8 - 24 h. After incubation the zones of inhibition were taken and isolate resistance to any of the 2^{nd} and 3^{rd} generation cephalosporins were further tested for ESBL production using double disc diffusion test. The antibiotics tested were ampicillin (25 µg), amoxicillin-clavulanic acid (30 µg), cefuroxime (30 µg), cephalexin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), nalidixic acid (30 µg), amikacin(30 µg), tetracycline (25 µg), gentamicin (10 µg), ofloxacin (5 µg) and ciprofloxacin (5 µg) (Oxoid UK).

Double disc synergy test (DDST)

Molten Mueller-Hinton agar plates were aseptically prepared and 0.1 ml of *E. coli* suspension equivalent to 0.5 MacFarland equivalent standard were inoculated on the surface of the Mueller-Hinton agar plate using a sterile swab sticks. A combination disc containing (amoxicillin, 20 µg and clavulanic acid 10 µg) was placed at the centre of the petri-dish and ceftaxidime (30 µg) and cefotaxime (30 µg) were placed 15 mm apart center to center on the plates. This was incubated at 37^0C for I8 - 24 h. An enhanced zone of inhibition between any one of the beta-lactam discs and the amoxicillin-clavulanic acid disc was interpreted as presumptive evidence for the presence of ESBL.

Agar dilution test

Antimicrobial susceptibility testing was done using the reference agar dilution method as described by the National committee for clinical laboratory standards (NCCLS, I999) against 31 clinical isolates of *E. coli* that were positive for ESBL. The quality control strain used *E. coli* ATCC 25922 and the antimicrobial agent tested were septrin, erythromycin, gatifloxacin, doxycycline, tetracycline, sparftoxacin, nitrofurantoin, ciprofloxacin, gentamicin, ofloxacin, pefloxacin, augmentin, ceftriaxone, ampiclox, ampicillin, streptomycin, amoxicillin sodium, ceftazidime, and cefuroxime.

RESULTS AND DISCUSSION

Out of one hundred and twenty three clinical isolates of *E. coli* screened for ESBL expression, 21 (17.0%) clinical isolates of *E. coli* from blood samples and 10 (8.1%) from blood samples express ESBL phenotypically. In all 31 (25.2%) of clinical isolates of *E. coli* from blood and urine produces ESBL enzymes. Ceftazidime gave the highest enhancement with augmentin thereby suggesting that the ESBL enzymes present phenotypically are TEM and SHV. The resistance rates of *E. coli* ESBL strains to septrin, erythromycin, tetracycline, doxycycline, cefotaxime, spafloxacin, nitrofurantoin, gatifloxacin, ciprofloxacin and gentamicin were 83.3, 90.3, 90.3, 83.8, 48.4, 48.4, 58.1, 6.5, 54.8 and 48.4% respectively (Figure 1). Figure 2 represents the resistance rates of *E. coli* ESBL strain were pefloxacin, Augmentin, ofloxacin, Ampiclox, ampicillin, streptomycin, amoxycillin sodium, ceftazidime, cefuroxime were 51.1, 9.7, 42.2, 87.1, 87.1, 87.1, 90.8, 61.3, and 74.2% respectively. In our study the resistances of ESBL positive organisms to these two antibiotics were highest with streptomycin (87.1%) an aminoglycoside, while the least resistance was observed with gatifloxacin (6.5%), a quinolone. The difference in resistance with other quinolones and aminoglycoside was not significant, while the overall result showed that ESBL producing isolates of *E. coli* are multi- drug resistant.

ESBL enzymes generally result from point mutations in the genes of broad – spectrum β-lactamase Ambler class A enzymes, such as TEM-1, TEM-2 or SHV-1. They are usually located on plasmids that often carry genes responsible for resistance to other antimicrobial agents, making it extremely difficult to treat infectious caused by bacteria that produce ESBL enzymes (Livermore, 1995). Our findings showed that ESBL producing organisms are multi-drug resistant and the organisms were resistant to all the antibiotics tested except gatifloxacin and augmentin that was active against them. Some studies have shown that treating infection caused by ESBL with cephalosporins often do not yield good therapeutic result and suggested that floroquinolones and aminoglycoside could be of an alternative choice (Albinu, 2003). ESBL isolates from our study were found to be resistant to virtually all the antibiotic except for gatifloxacin (a quinolone) and augmentin (an aminoglycoside). Imipenem, cefoxitin are known to be active against ESBL producing organisms but in our country we have the problem of unavailability or when available it is expensive and most patients cannot afford it. Quinolones and aminoglycoside are two alternative antibiotics that are always available here, which can be use against ESBL infection but our findings do not show total susceptibility of ESBL positive organisms to these two antibiotics. *E. coli* is a member of the family Enterobacteriaceae that is an important group in community and hospital-acquired infections and patients in the intensive care unit, those that are receiving invasive treatment and those that have stayed long in the hospital are at high risk of infections caused by *E. coli*. Unfortunately, resistance has become increasing common with *E. coli* expressing ESBL thereby making empiri-

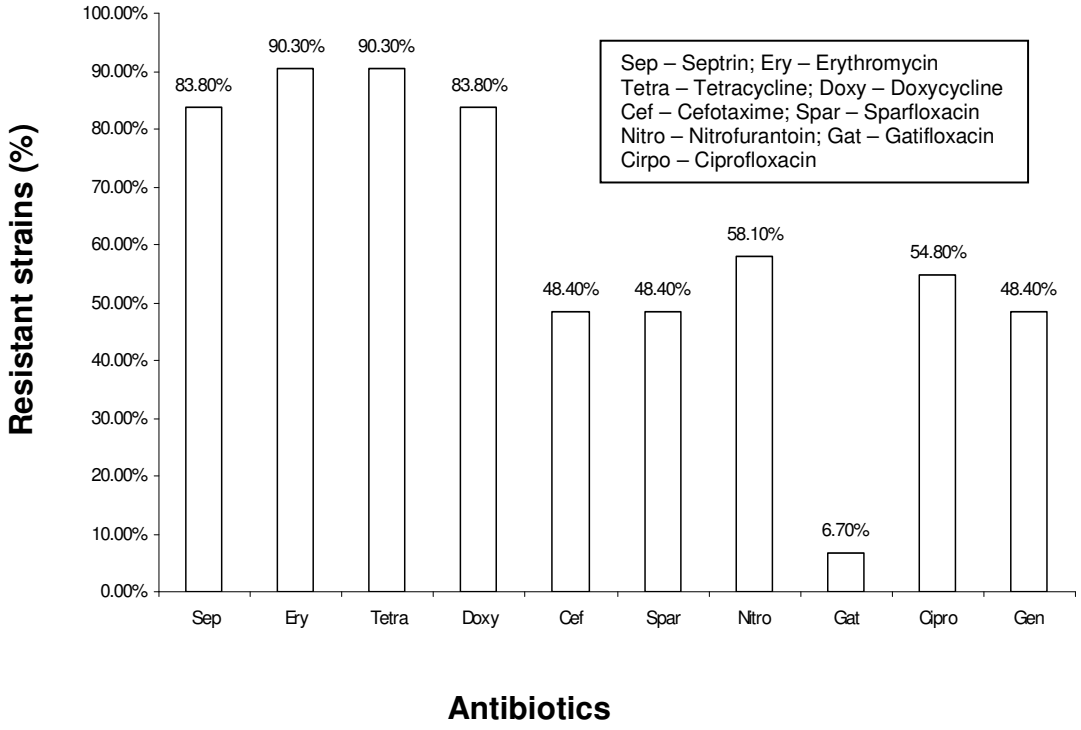

Figure 1. Antimicrobial resistance of 31 strains of *E. coli* to septrin, erythromycin, tetracycline, doxycycline, cefotaxine sparfloxacin, nitrofurantoin, gatifloxacin, ciprofloxacin and gentamicin.

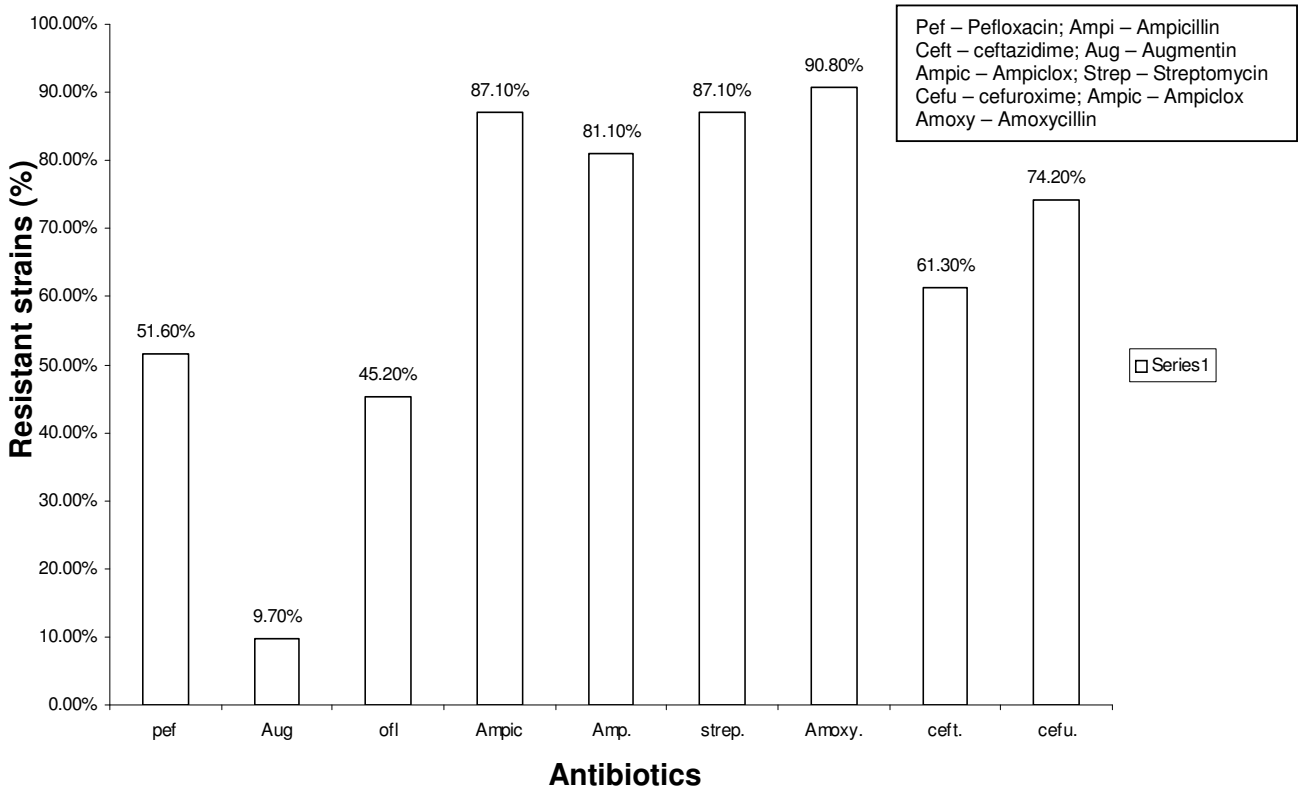

Figure 2. Antimicrobial resistance of 31 strains *E. coli* to pefloxacin, augmentin, ofloxacin, ampiclox, ampicillin, streptomycin, amoxycillin sodium, ceftaxidime and cefuroxime.

cal therapy decisions more difficult (Yan et al., 2000). The most serious resistance patterns now-emerging among Gram-negative organisms include resistance to extend-pectrum cephalosporins and penicillins as a result of constant use of these agents in our medical institutions (Diekema, 1999). This resistance is commonly mediated by ESBLs in *E. coli* and *Klebsiella* species, or by the hyper-production of chromosomally mediated cephalo-sporinases in *Citrobacter, Serratia* and *Citrobacter* species (Cars et al., 2001).

Based on our findings, we can suggest the use gatifloxacin and augmentin as an effective alternative in the treatment of ESBL infections caused by *E. coli.* Hospitals should have proper policies and guidelines for the prudent use of antimicrobial agents in order to reduce or discourage the emergence and spread of ESBL producing organisms. Since these ESBL strains easily develop resistance to various antibiotics, which can spread rapidly, appropriate and efficient infection control measures should be developed, followed, and practiced by all health and non-health care staffs in order to checkmate resistance out break. Another factor that could be responsible for increase resistance of ESBL is that some clinicians are unaware of ESBL producing isolates and as a result could not report them. Because they fail to report them, the patients keep harbouring and spreading these enzymes from patient to patient and from hospital to hospital as a result of treatment failures (Tenover et al., 1999).

In conclusion antibiotic resistance is on the increase globally especially among Gram-negative bacteria. Therefore, there is a need for periodic antibiotic resistance survey to help orient physicians and the local population on the best treatment strategies. NCCLS should come up with a standard technique that will be easy and fast in detecting ESBL from both *Enterobactericeae* and other organism. They should also create awareness to both the clinician and the local population about the existence of ESBL and we suggest that more research should be embarked on in search of an effective alternative antibiotic that can be used in treating infections caused by ESBL producing organisms.

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