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Detection of Cefotaximase Genes of Beta Lactamase among Clinical Isolates of *Escherichia coli* in a University Teaching Hospital, Nigeria

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Cefotaximase genes of Beta Lactamase have been responsible for community acquired infections such as urinary tract infections. Presently, organisms harbouring these genes are spreading across hospitals world wide. The presence of these genes among *Escherichia coli* has posed serious therapeutic problems. In this study, cefotaximase genes of beta lactamase among clinical isolates of *Escherichia coli* were detected using polymerase chain reaction. We also investigated the antibiotic susceptibility pattern of this organism from June-November 2012. Clinical isolates from patients were collected at the bacteriology laboratory of a university teaching hospital. The isolates were subjected to proper identification using standard microbiological techniques. Antibiotic susceptibility testing was done using the Disc Diffusion Method. Amongst the isolates of *E. coli* collected, 100% susceptibility to one or more antimicrobial agent(s) by all the isolates was not observed. An average of 30-65 isolates of *E. coli* showed intermediate susceptibility to complete resistance to all the antimicrobial agents. It is of clinical interest that the *E. coli* susceptibility pattern observed in this study is incorporated in the available antibiotic susceptibility profile of *E. coli*. However, high rate of resistance to third generation cephalosporins, aztreonam and ciprofloxacin observed was disturbing. Isolates harbouring Cefotaximase (CTX-M) genes showed more resistance to cefotaxime. There was 53% prevalence rate of Cefotaximase gene among the isolates. Nevertheless, the continuous monitoring of antibiotic susceptibility pattern of *E. coli* in Nigerian hospitals is needed to assess the challenge regarding drug resistance, which will allow doctors to adjust the empirical treatment.

Key words: *Escherichia coli*, antibiotic susceptibility, clinical isolates, cefotaximase genes

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INTRODUCTION

Escherichia coli is a clinically important pathogenic Gram negative bacteria that causes infections such as Urinary Tract Infections (UTIs), pneumoniae, and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases. Resistance to antimicrobial drugs commonly used for the treatment of *E. coli* infections have been reported (Opal and Pop-Vicas, 2010). This has posed a serious challenge in the treatment of *E. coli* infections (Murray *et al.*, 2007).

Beta lactamases are enzymes that hydrolyze beta lactam antibiotics like penicillins and cephalosporins (Paterson and Bonomo, 2005). CTX-M beta lactamase hydrolyzes mostly cefotaxime, a third generation cephalosporin (Lee *et al.*, 2009). They are commonly found in the Enterobacteriaceae family (Kevin, 2006). CTX-M is prevalent in community acquired urinary tract infection. With the spread of these genes in hospitals all over the world, it is necessary to know their prevalence in a hospital so as to formulate a policy of empirical therapy in high-risk units (Canton and Coque, 2006). Studies have shown their rapid increasing prevalence in hospitals. In Nigeria, CTX-M was found to be prevalent at the Federal Medical Centre, Yola (Isaiah *et al.*, 2011). Bacteria harbouring Cefotaximase genes are common causes of infections among community-dwelling persons without a history of hospitalization. Thus, these organisms can then be introduced into hospitals (Pitout and Laupland, 2008).

Variation in susceptibility is great and laboratory tests for antimicrobial susceptibility are essential. Therefore, in choosing the type of therapy for *E. coli* infections, it is important to avail of information on the antibiotic susceptibility pattern of the isolates (Yu *et al.*, 1999). The research studies on Cefotaximase genes of Beta lactamases from Nigeria are few. Therefore, the aim of this study was to detect the presence of cefotaximase (CTX-M) genes of beta lactamase among clinical isolates of *E. coli* from a University Teaching Hospital in Anambra State, Nigeria.

MATERIALS AND METHODS

One hundred and fifty properly identified *E. coli* isolates from clinical specimens: Urine (65), Wound Swab (28), Vaginal Swab (32) and Sputum (25) in the routine bacteriology laboratory of a University Teaching Hospital, Anambra State, Nigeria was collected for this work.

Isolates were identified using microscopy, biochemical testing and culturing in appropriate media.

Pure cultures of the isolates were stored in nutrient agar slant at 4°C for further analysis.

Antimicrobial susceptibility testing: The antimicrobial agents used were: Cefotaxime (30 µg), Ceftazidime (30 µg), Augmentin (30 µg) (Amoxicillin 20 µg/clavulanic acid combination 10 µg), Ciprofloxacin (5 µg), (From Abtek Biological Ltd, Liverpool, UK), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Fosfomycin (50 µg) and Aztreonam (30 µg) (From Oxoid, UK). A suspension of the test organism was prepared to turbidity equivalent to 0.5 McFarland standards and an aliquot was inoculated on Muller Hinton agar plate using sterile swab stick. All plates were incubated for 18-24 h at 37°C in air. Antimicrobial susceptibility testing was performed on each isolate by disc diffusion method and diameter of zones of inhibition were interpreted as; Susceptible (S), Intermediate (I) and Resistant (R) as recommended by Clinical Laboratory Standard Institute (CLSI, 2011). *E. coli* ATCC 25922 was used as control.

Screening for extended spectrum beta lactamase (ESBL): All the isolates were screened for Extended Spectrum Beta-lactamase (ESBL) by the Double Disk Synergy Test (DDST). A suspension of the test organism was prepared to turbidity equivalent to 0.5 McFarland Standards and was inoculated on Muller Hinton agar plate. A disc containing Amoxicillin plus Clavulanic acid (moxclav 20/10 µg) disc was placed centrally on the Muller-Hinton agar plate. Discs containing Ceftazidime (30 µg) was placed 15 mm out from the edge of moxclav disc. The same was performed with Cefotaxime (30 µg). Plates prepared were incubated at 37°C, aerobically for 18-24 h zone-enhancement toward moxclav disc was recorded for all the cephalosporins, as per Clinical Laboratory Standard Institute (CLSI) guidelines. Plates with negative result were further incubated at 37°C, aerobically for 18-24 h. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control, respectively.

DNA extraction and detection of cefotaximase gene: ESBL positive isolates were subjected to polymerase chain reaction and plasmid DNA was extracted according to the published method of Johnson and Woodford (1998). Specific primers for amplifying the *bla*CTX-M genes by PCR were CGC TTT GCG ATG TGA AG and ACC GCG ATA TCG TTG GT. Annealing Temperature was 63°C.

PCR was carried out in solution containing 200 µM concentration of dNTPs, 10 Pmol of each primer, 1.5 mM µL⁻¹ MgCl₂, 1U *Taq* polymerase and 2 µL⁻¹ DNA

template in a final volume of 50 μ l; were analyzed using gel electrophoresis in a 2% (W/V) agarose gel. *Escherichia coli* 6681 containing *bla*_{CTX-M} gene was used as control.

Statistical analysis: Parametric methods (unpaired t-test procedure using Microsoft excel) were used for statistical analysis of the data obtained from drug susceptibility testing. While, standard percentage occurrence for the calculations of data received.

RESULTS

From this study, *Escherichia coli* showed highest prevalence (150) among other gram negative organisms-*Klebsiella pneumoniae* (115), *Pseudomonas aeruginosa* (65) and *Enterobacter* spp (40) isolated from the clinical specimens. The susceptibility profile showed 10.8% susceptibility of the isolates to Ceftazidime, 20% susceptibility to Cefotaxime, 35.4% susceptibility to Cefepime (Table 1). The resistance patterns across the antimicrobial agents are: Co-amoxiclav (33.8%), Cefepime (64.6%), Ciprofloxacin (66.2%), Aztreonam (72.3%), Ceftazidime (75.4%) and Cefotaxime (75.4%). (Table 1) showed the results of percentage of intermediate pattern across the antibiotics in the isolates.

It was observed that following detection of ESBL isolates of *Escherichia coli*, a prevalence rate of 83% emerged. The prevalence of the enzyme across the various clinical specimens showed that the ESBL-producing isolates recovered from Urine were the highest (Table 2). The prevalence of *bla*_{CTX-M} genes across the various clinical specimens was 53% with distribution across the clinical specimen as thus: Urine (29), Wound Swab (15) and Sputum (9) (Table 2).

Table 1: Antibiotic Susceptibility of all the *E. coli* isolate

| Antimicrobial agents | Susceptible (%) | Intermediate (%) | Resistant (%) |
|---------------------------|-----------------|------------------|---------------|
| Meropenem (10 μ g) | 92.3 | 4.61 | 3.1 |
| Imipenem (10 μ g) | 80 | 12.3 | 7.7 |
| Fosfomycin (50 μ g) | 75.4 | 7.7 | 16.9 |
| Cefotaxime (30 μ g) | 20 | 4.6 | 75.4 |
| Ceftazidime (30 μ g) | 10.8 | 13.8 | 75.4 |
| Aztreonam (30 μ g) | 23.1 | 4.6 | 72.3 |
| Co-amoxiclav (30 μ g) | 21.5 | 44.6 | 33.8 |
| Cefepime (30 μ g) | 35.4 | 0.0 | 64.6 |
| Ciprofloxacin (5 μ g) | 26.2 | 7.7 | 66.2 |

Table 2: Prevalence of ESBL and *bla*_{CTX-M} gene producing *Escherichia coli* among the clinical specimens

| Clinical specimen | No. of <i>E. coli</i> isolates | No. of ESBL isolates | No. of isolates with <i>bla</i> _{CTX-M} gene |
|-------------------|--------------------------------|----------------------|---|
| Urine | 65 | 57 | 29 |
| Vaginal Swab | 32 | 26 | 13 |
| Wound Swab | 28 | 23 | 15 |
| Sputum | 25 | 18 | 9 |
| Total | 150 | 124 | 66 |

DISCUSSION

In this study, *E. coli* had the highest prevalence among other gram negative bacteria. This shows a high prevalence of *E. coli* associated infections among patients in this hospital. This work justifies other studies done in Nigeria and other parts of the world which showed that *E. coli* is the most prevalent pathogenic organism in the hospital environment.

The antibiotic susceptibility pattern observed in this study reveals multi- drug resistance among *E. coli* isolates. This may reflect the ability of *E. coli* to acquire antimicrobial resistance in the face of new and previously used antimicrobial agents. With an average of 55 susceptible isolates, 30 intermediate isolates and 65 resistant isolates; we are sure to have serious therapeutic problems with the treatment of *E. coli* associated infections.

Multi- drug resistance is common and is under the control of transmissible plasmids. These transmissible plasmids frequently carry genes encoding resistance to several drug classes (Ryan and Ray, 2004). This may explain the presence of CTX-M gene among the isolates as well as other multi- drug resistant genes that were not investigated in this study. These genes could be responsible for the multi- drug resistance observed in this study.

The distribution of CTX-M genes in this study justifies studies done by Isaiah *et al.* (2011) in Yola, Nigeria and Iroha *et al.* (2012) in Enugu, Nigeria. Their studies revealed the presence of CTX-M genes in hospitals. This work therefore provides a second report on CTX-M genes among *E. coli* in South Eastern Nigeria. Treatment of infections caused by *E. coli* has no single specific therapy available (Giske *et al.*, 2008). Variation in susceptibility is great and laboratory tests for antimicrobial susceptibility are essential. Therefore, in choosing the type of therapy for *E. coli* infections, it is important to avail of information on the prevailing levels of antimicrobial susceptibility from the isolates (Yu *et al.*, 1999).

In conclusion, the prevalence rate of Cefotaximase producing organisms of beta lactamase is high globally. These organisms are known to cause serious nosocomial infections, long term carriage in the community, community-acquired infections such as urinary tract infections. The findings from this study revealed high prevalence of Cefotaximase genes from Nnamdi Azikiwe University Teaching Hospital (NAUTH) amongst *E. coli* having a high prevalence of 53%. This certainly calls for urgent attention of the various parastatals in the Ministry of Health as well as hospital administrators. This study

thus, emphasizes the need of enhanced infection control in hospitals and clinics, especially in the developing countries to limit the spread of these strains.

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