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The Bacterial Isolates and Plasmid Profile of Extended Spectrum Beta-Lactamases Producers Causing Urinary Tract Infection among Pregnant Women in Uyo, Nigeria

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Abstract

Background: Extended spectrum beta-lactamases (ESBLs) are enzymes that compromise the efficacy of all beta-lactams and are spread by plasmids. They are of public health importance the world over; however, in Nigeria in general and Uyo in particular, tests for their detection are not routinely done in hospital laboratories despite increase in treatment failures observed for common clinical conditions like urinary tract infection. Objective: To isolate ESBLs producing uropathogens and the plasmid underlying their resistance to antibiotics. Materials and Methods: Three hundred urine specimens (n = 300) were collected from pregnant women attending antenatal clinics at St. Lukes Hospital, Anua, cultured and incubated according to accepted standard. Identification of isolates was done using Microbact 24E (Oxoid, UK) system. The predominant bacterial pathogens were *Escherichia coli* (42%) followed by *Klebsiella pneumonia* (21%), *Klebsiella oxytoca* (12%), *Citrobacter* spp. (5%), *Proteus mirabilis* (7%), *Enterobacter* spp. (12%) and *Acinetobacter baumanii* (1%). The isolated bacteria were tested for their antibiotic susceptibility using Clinical Laboratory Standard Institute (CLSI) recommended disc diffusion method. A Double Disk Synergy Test (DDST) and Phenotypic Disk Confirmatory Test (PDCT) were performed to determine ESBL production. Chromagar ESBL was also used to test for the presence of ESBL producing isolates. The plasmid content of ESBL producing isolates and their participation in drug resistance were investigated. Results: Of the 80 bacterial isolates causing urinary tract infection in these women, the ESBL producers were found to be 16 (20%). Out of these 16 ESBL producing urogenital isolates *Klebsiella pneumonia* (8, 50%) was the most prevalent. Others include *Escherichia coli* (38%), *Klebsiella oxytoca* (6%) and *Enterobacter cloacae* (6). Plasmid content of ESBL producing isolates was found to be 87.5%. Conclusion: The Extended Spectrum Beta-lactamase producing uropathogens mainly of plasmid origin are increasingly responsible for the cause of community acquired urinary tract infections in pregnant women in Uyo.

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Keywords

ESBL, Plasmid Content, Uropathogens

1. Introduction

Urinary tract infection is defined as multi-action of organisms in the urinary tract with the presence of $>10^5$ organisms per milliliter in mid-stream urine [1]. The organisms causing UTI in community include *Escherichia coli* which is the commonest, Proteus spp., *Klebsiella* spp., *Pseudomonas* spp. and *Streptococci* spp. [2].

Pregnant women are at risk of urinary tract infections beginning in week 6 and peaks at weeks 22 to 24 [3]. The three clinical manifestations of UTIs in pregnancy are asymptomatic bacteriuria, acute cystitis and pyelonephritis [4]. Abortion, small birth size, maternal anaemia, hypertension, preterm labour, phlebitis, thrombosis, and chronic pyelonephritis are related to urinary tract infection during pregnancy [5].

Gram negative bacteria have the potential not only to develop chromosomal resistance but also to quickly spread resistance via genetic determinants carried on plasmids or transposons and rapid spread through human travel and migration. These resistant elements have created a global treatment crisis. Multi drugs resistance is increasingly seen in many Gram negative bacteria as a result of wide spread use of various antibiotics [6].

Extended spectrum Beta-lactamases (ESBls) are enzymes that compromise the efficacy of all beta-lactams, except Cephamycins and Carbapenems by hydrolysis of beta-lactam ring and are inhibited by beta-lactam inhibitors [7]. Resistant bacteria are a big threat to hospitals and community [7]. Among the wide array of antibiotics, beta-lactams are the most commonly used agents accounting for over 50% antibiotics in use [7]. There are various types of ESBLs derived mainly from the groups TEM, SHV, and CTX-M variants. This extended-spectrum β-lactamases contribute to multidrug resistance among the organisms and detection of these enzymes is crucial to treatment. Their detection is therefore significant in preventing treatment failures caused by these pathogens.

Plasmids are linear but usually circular replicons of extra chromosomal DNA in bacteria, and they play an important role in the evolution of microbes [8]. The genes encoding ESBLs are usually located on plasmids that are highly mobile and can harbor resistance genes to several other unrelated classes of antimicrobials [9], such as the plasmid-mediated quinolone resistance gene and aminoglycoside resistance gene [10].

2. Methodology

Bacterial isolation: All urine samples (first morning urine) were aseptically inoculated unto MacConkey and blood agars using the calibrated loop technique. After overnight incubation at 37˚C for 24 hours, colony counts yielding bacterial growth of 10^5/ml of urine were regarded as significant for bacteriuria. Identification of bacterial isolates was carried out using Microbact 24E (MB24E).

ESBL detection: For the Phenotypic Disk Confirmatory Test (PDCT), all isolates showing inhibition zone size of ≤22 mm with ceftazidime (30 µg), ≤25 mm with cefotazime (30 µg), ≤27 mm with Azetronam (30 µg) and ≤22 mm with cefpodoxime (10 µg) was identified as potential ESBL. A Double Disk Synergy Test (DDST) was done in which case, Ceftazidime 30 µg disc was placed on Mueller Hinton sensitivity plate 20 mm (center to center) from Augumentin 30 µg disk (amoxicillin and clavulanate 20 µg/10 µg). After incubation for 18 - 24 hours at 37˚C a clear extension of the edge of ceftazidime disc inhibition zone towards the disk containing clavulanate is described as synergy indicating the presence of an ESBL. Furthermore, gram negative bacterial isolates were cultured on Chromagar which is a chromogenic medium designed specifically for the Screening of Extended Spectrum β-Lactamase-producing *Enterobacteria* (ESBL) [11]. The antibiotic discs were from OXIOD England, and the various methods of ESBL detection used were for comparison and quality control. Plasmid DNA Isolation and Gel electrophoresis: Subculture was done on Tryptone Soya Agar and a horizontal electrophoresis apparatus were applied following standard procedures for the isolation of plasmid DNA and electrophoresis respectively [12]. The data obtained were analysed statistically using the chi square to determine the relationship between the variables and its significance.

3. Results

The 300 urine samples from different age groups of pregnant women (**Table 1**), yielded 80 clinical isolates including *Escherichia coli* (42%) followed by *Klebsiella pneumoniae* (21%), *Klebsiella oxytoca* (12%) *Citrobacter* spp. (5%), *Proteus mirabilis* (7%), *Enterobacter* spp. (12%) and *Acinetobacter baumanii* (1%) were obtained (**Table 2**).

The isolates were screened for ESBL production and 16 (20%) were found to be ESBL producers by the DDST method viz; *Klebsiella* spp. (56%), *Escherichia coli* (38%), and *Enterobacter cloacae* (6%) as shown in **Table 3**. The PDCT and the Chrom agar ESBL test methods revealed more numbers of ESBL producers at 47% and 70% respectively (**Table 3**). Some strains of *Klebsiella pneumoniae* especially *K. pneumoniae*-3 and 5 were not of plasmid origin (**Table 4**). The Agarose gel electrophoresis of plasmids recovered from the ESBL isolates is as shown in **Figure 1**.

Table 1. Distribution of bacterial isolates among the pregnant women.

Age group (yrs)	No. of samples (%)	Number of isolates (%)
15 - 24	100 (33.3)	27 (33.7)
25 - 34	157 (52.3)	45 (56.3)
35 - 44	43 (14.3)	8 (10.0)
Total	**300 (100)**	**80 (100)**

Table 2. Frequency of bacterial isolates.

Bacterial isolates	Total No. (%)
Eschericha coli	32 (40)
Klebsiella pneumonia	16 (20)
Klebsiella oxytoca	9 (11)
Citrobacter sakazakii	2 (3)
Citrobacter freundii	2 (2)
Proteus mirabilis	1 (1)
Enterobacter spp.	11 (14)
Acinetobacter baumanii	7 (9)
Total	**80 (100)**

Table 3. ESBLs detection by PDCT, DDST and Chrom agar ESBL.

Bacterial isolates	Total n (%)	PDCT n (%)	DDST n (%)	Chrom Agar ESBL n (%)
E. coli	32 (40)	19 (40)	6 (38)	32 (46)
Klebsiella spp.	25 (32)	13 (28)	9 (56)	25 (35)
Citrobacter spp.	4 (5)	1 (2)	0 (0)	4 (6)
P. mirabilis	1 (1)	1 (2)	0 (0)	0 (0)
E. cloacae	11 (14)	7 (15)	1 (6)	9 (13)
A. baumanii	7 (9)	6 (13)	0 (0)	0 (0)
Total	**80 (100)**	**47 (100)**	**16 (100)**	**70 (100)**

Chi square test, the level of statistical significance was $p < 0.05$. Key: DDST: Double Disk Synergy Test, PDCT: Phenotypic Disc Confirmatory Test.

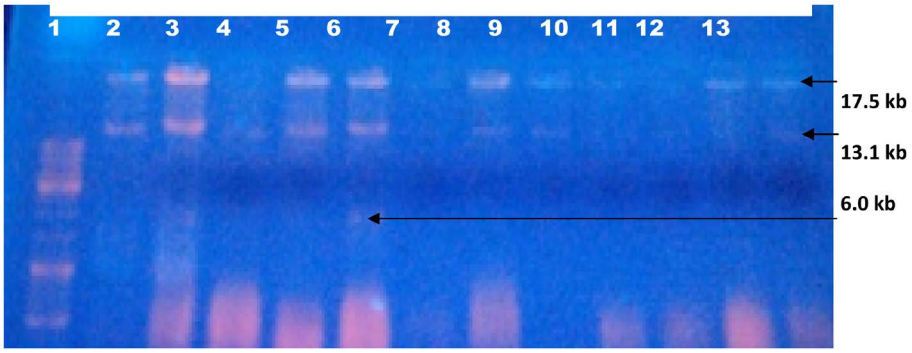

Figure 1. Agarose gel electrophoresis of plasmids recovered from the ESBL isolates. Lane 1. 10 kb DNA ladder; Lanes 2-8 = *K. pneumoniae* 02-08; Lanes 9 = *Enterobacter cloacae* 01; Lanes 10-13 = *E. coli* 02.

Table 4. Plasmid profiles of ESBL producing isolates.

Bacterial Isolates	Plasmid size, kb
K. Oxytoca	17.5, 13.5
E. coli-1	17.5 13.5
E.coli-2	17.5, 13.5, 8.0, 6.0
E.coli-3	17.5, 13.5
E. coli-5	17.5, 13.5
E. coli-6	17.5, 13.5
K. Pneumoniae-1	17.5, 13.5, 8.0
K. Pneumoniae-2	17.5, 13.5
K. Pneumoniae-3	-
K. Pneumoniae-4	17.5, 13.5
K. Pneumoniae-5	-
K. Pneumoniae-6	17.5, 13.5
K. Pneumoniae-7	17.5, 13.5
K. Pneumoniae-8	17.5, 13.5
K. Pneumoniae-9	17.5, 13.5
E. cloacae	17.5, 13.5

4. Discussion

This study revealed 80 positive cultures out of the 300 urine samples or 26.6% prevalence rate of Gram negative bacteria isolates as a cause of Urinary tract infection (UTI) in pregnancy in Uyo. Although Escherichia coli was found to be the most common cause, studies from some Cities in Nigeria and other African countries has reported higher rates of urinary Gram negative bacterial isolates of up to 51.0%, 67.5% and 61.9% in Rivers state Nigeria, Ethopia and Tanzania respectively [13]-[15]. The lower percentage could be attributed to standard sample collection and automated biochemical method of bacterial identification employed in this study as against the conventional method of bacterial identification used in these other studies. Of note also is the higher rate of urinary tract infection 45 (56.3%) among women of age bracket 25 - 34 years. This may be attributed to the fact that most women at this age are likely to have gotten married and would want to have children as revealed by their more participation in this study.

Extended-spectrum beta lactamase producing uropathogens are of public health concern because of their ability to express resistance to antibiotics used in treating urinary tract infections. In this study, the commonly used DDST method of assessing ESBL showed a total prevalence of 20%. This agrees with some studies which showed prevalences between 22% and 27.7% [16]-[19]. However, other methods used in this study (PDCT and Chrom agar) used for the detection ESBL revealed higher overall and individual isolates prevalences. This may be due to the higher sensitivities of these methods in detecting ESBL. Some recent studies reported significantly

higher prevalence of ESBL UTI [20] reported a prevalence of 59.6% respectively.

Fourteen out of the 16 ESBL producing isolates harboured plasmids; 2 isolates did not contain plasmid. This could be due to the fact that many of the genera of gram negative bacteria possess a naturally occurring chromosomally mediated beta-lactamases [9]. This result agrees with the findings of [21]. The involvement of plasmids as a factor responsible for antibiotic resistance in the ESBL isolates further suggests the emergence and active transfer of antibiotic resistance and R plasmids among the circulating strains causing ESBL UTI in Uyo.

5. Conclusion

The extended spectrum beta-lactamases producing uropathogens mostly of plasmid content are responsible for community acquired urinary tract infection among pregnant women in Uyo. The detection for ESBL is not routinely requested or tested. It is therefore important to change this practice to prevent the treatment failures caused by these pathogens.

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