



Research Article

Prevalence and molecular analyses of extended spectrum β -lactamase producing uropathogens among pregnant women, Jigawa state, Nigeria

Eze L Chinyere^{1*}, Sani M Nura¹, Gumel M Ahmad¹, Amoo F Kemi¹ and Mujahid N Sani²

¹Department of Microbiology and Biotechnology, Federal University Dutse, 7156 Jigawa State, Nigeria

²Department of Microbiology, Kano University of Science and Technology, Wudil Kano, Nigeria

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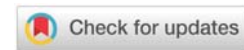
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*Corresponding author: Eze L Chinyere, Department of Microbiology and Biotechnology, Federal University Dutse, 7156 Jigawa State, Nigeria, Tel: 08068535692; E-mail: lovelyneze50@gmail.com

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Abstract

The Extended Spectrum β -Lactamase (ESBL)-producing uropathogens are of serious clinical concern worldwide. They are plasmid-mediated enzymes that are capable of hydrolysing virtually all β -lactam antibiotics including oxyimino-cephalosporins and monobactams. In this study, the uropathogens and risk factors for Urinary Tract Infections (UTI), their antibiotic susceptibility patterns, and plasmid studies of ESBL-producing uropathogens prevalent in pregnant women attending to Rasheed Shekoni Teaching Hospital (RSTH) and Dutse General Hospital (DGH) were investigated. During the current work, midstream urine samples were collected aseptically from 200 pregnant women and then cultured. The antibiotic susceptibility testing and ESBL detection were done using the standard methods. Identification of the recovered bacterial pathogens were carried out using the standard procedures and then confirmed through molecular analyses. The prevalence of UTI and ESBL production in this study was 37.0% and 17.6%, respectively. In pregnant women, the prevalence of UTI was higher in DGH (27.0%) than in RSTH (10.0%). The most predominant isolated uropathogens were *Alcaligenes* sp. (58.1%) followed by *Klebsiella pneumoniae* (28.3%). The most sensitive antibiotic was ceftazidime (31.2%). Molecular enumeration using 16S rRNA partial sequencing revealed the presence of 750bp. There is an urgent need to enlighten the pregnant women on the hazardous effects of self-medication, over-the-counter prescription, possible risk factors of UTI and the importance of personal hygiene during antenatal clinic, in order to prevent the emergence and spread of antibiotic resistant bacteria.

Introduction

Beta-lactamases are enzymes produced by bacteria that breakdown a particular class of antibiotics called the beta-lactams. As the bacteria developed resistance to one type of beta-lactam antibiotic, new antibiotic derivatives (cephalosporins, carbapenems and monobactams), which in turn made the bacteria to continually evolved and developed new set of enzymes described as extended-spectrum beta-lactamases (ESBLs) that can break down the newer derivatives. ESBLs are plasmid mediated enzymes that confer resistance to penicillin, third generation cephalosporins and aztreonam but are said to be inhibited by clavulanic acid [1]. The global

increase in antibiotic resistance among these uropathogens are due to their ability to hydrolyse virtually all the beta-lactam antibiotics including the 3rd generation cephalosporins and carbapenems by producing ESBLs such as CTX-M enzymes (CTX-M-14 and CTX-M-15) or AmpC β -lactamases and OXA-type beta-lactamases [2,3].

In Nigeria, there have been reports on multi-drug resistance and various infections with ESBL producing organisms including recent study on plasmid profile of ESBL producing bacterial isolates causing urinary tract infection among pregnant women in Uyo [4].

ESBL producing bacteria such as *E. coli*, *Klebsiella sp*, *Proteus sp* and *P. aeruginosa*, have been found to continuously develop resistance to several antibiotics, which has resulted to wide spread cases of chronic urinary tract infections (UTI) in pregnant women. It has been widely reported that the bacteria harbour antibiotic resistant genes which can be horizontally transferred to other bacteria thus call for urgent research on their prevalence and management [5].

This study, intend to report on the prevalence and molecular typing of ESBL uropathogens among pregnant women, thereby providing an avenue for policy makers and researchers to build on effective management policies and public health strategies.

Materials and methods

Study site

This study was carried out at the Rasheed Shekoni Teaching Hospital and Dutse General Hospital, Jigawa State, Nigeria. According to National Population Commission, Jigawa state is one of the developing cities in the North-west of Nigeria, and is one of the less populated state in the Northern region.

Study population

The studied populations include samples from 200 pregnant women randomly collected attending Rasheed Shekoni Teaching Hospital and Dutse General Hospital Jigawa State, Nigeria. About 100 samples were collected from each hospital individually.

Samples collection

Structured questionnaires were used to collect demographic and clinical information from all patients. Clean catch midstream urine was aseptically collected and processed immediately into the Microbiology laboratory.

Specimen processing and isolation of the uropathogens

Laboratory analysis of the urine samples including macroscopic examination was conducted, to check out the color, turbidity and presence of blood. The uncentrifuged urine samples were mixed by rotating the plastic container before inoculating the surface of MacConkey agar and nutrient agar, followed by incubation at 37°C for 24 h [6].

Identification and characterization of the bacterial isolates

Further studies and identification of the bacterial isolates was based on colony morphology, Gram staining, and biochemical characterization using standard method [6].

The antibiotic susceptibility tests

The antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion method; isolated colonies was inoculated in Muller Hinton Agar plate and spread evenly over the surface, then the disc of commercially available discs (Oxoid Uk)) with standard antibiotic concentrations of

Ceftazidime (30µg), Cefotaxime (30µg), Tetracycline (30µg), Augmentin (30µg), Ofloxacin (5µg) and Gentamicin (10µg) were placed on the culture plate and incubate at 37°C. After 24h the zone of inhibition around the discs were observed following recommendations of the Clinical and Laboratory Standard Institute [7].

Determination of β-lactamase production by the bacterial isolates

The double-disc synergy test was used for confirming the extended spectrum β-lactamase (ESBL) production by the bacterial isolates. Augmentin (Amoxicillin/ clavulanic acid) was placed in the center of ceftazidime and ceftriazone in muller hinton agar plate inoculated with the bacterial strain, the plates were incubated at 37°C for 24h. The antibiotics used were cell wall synthesis inhibitors. The isolates showing inhibition zone diameter of ≤ 22 mm with Ceftazidime (30 µg), ≤ 27 mm with Ceftriazone (30 µg) and ≤ 27 mm with Augmentin (30 µg) were identified as potential ESBL producers [7].

Molecular analysis

These isolates were subjected to molecular characterization using 16S rRNA amplification and sequencing. The product of PCR amplicons was separated using gel electrophoresis. The sequence was parsed for genomic analogy using EMBL Blastn database, in reference to Sun [8]. The MEGA-X software was used to construct the phylogenetic neighbor joining tree for species relationship. In addition, the evolutionary distance was established using bootstrapping.

Statistical analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS 20), all observed data was recorded as ±SEM (Standard Error of Means). Statistical significant established at 95% confidence interval $P < 0.05$.

Results

The prevalence of UTI among the pregnant women was found to be 37.0%.

Demographic data of patient in relation to UTI

The prevalence of UTI is highest in DGH 54(27.0 %) than in RSTH 20(10 %), as clear in (Table 1). The age group UTI distribution within the studied population showed that the prevalence is lowest (4.5 %) in age group of 31-40, and highest (27.5 %) in age group of 21-30. Results showed that there is no statistical significance difference in the prevalence of UTI($P < 0.05$) within the age group ($X^2 = 5.759$; $P = 0.056$). However, highest incidence of UTI is recorded in second trimester (19.0 %) followed by first trimester (10.0 %), and is least in the third trimester (8.0%).

Prevalence of ESBL production among the bacterial uropathogens

The recorded prevalence of UTI among the pregnant women is 74(37.0%) presented in Table 2. *Alcaligenes sp.* (58.1%) is the

**Table 1:** Demographic data of patients in relation to UTI.

Variables	Positive (%)	Negative (%)	OR	χ^2	P value
Address					
DGH	54(27.0)	46(23.0)	0.213	24.796	<0.001
RSTH	20(10.0)	80(40.0)			
Age					
<20	10(5.0)	34(17.0)	5.759	0.056	
21-30	55(27.5)	74(37.0)			
31-40	9(4.5)	18(9.0)			
Employment					
No	48(24.0)	78(39.0)	0.880	0.175	0.675
Yes	26(13.0)	48(24.0)			
Trimester					
1 st	20(10.0)	40(20.0)	0.543	0.762	
2 nd	38(19.0)	62(31.0)			
3 rd	16(8.0)	24(12.0)			
Parity					
Multigravida	50(25.0)	71(35.5)	0.620	2.455	0.117
Primigravida	24(12.0)	55(27.5)			
History of UTI					
No	65(32.5)	103(51.5)	0.620	1.287	0.257
Yes	9(4.5)	23(11.5)			

Where; UTI: Urinary Tract Infections; OR: Odd Ratio; χ^2 : Chi Square

Table 2: Prevalence of ESBL production among the bacterial uropathogens.

Bacterial isolates (N=74)	Number (%)	ESBL producers (%)
GRAM NEGATIVE		
<i>Alcaligenes</i> sp.	43 (58.1)	8 (18.6)
<i>K. pneumoniae</i>	21 (28.3)	4 (19.0)
<i>Myroides</i> sp.	6 (8.1)	1(16.7)
GRAM POSITIVE		
<i>S. aureus</i>	4(5.4)	0
Total	74(37.0)	13(17.6)

Where; *K. Pneumoniae*: *Klebsiella pneumoniae*; *S. Aureus*: *staphylococcus aureus*

predominant one followed by *K. pneumoniae* (28.3%). Of the total of 74 isolates, only 17.6% are ESBL producers.

Susceptibility pattern of the isolated uropathogens to the selected antibiotics

The antibiotic susceptibility assay revealed the increased resistance of the uropathogens against Ofloxacin, Gentamicin and Tetracycline (Table 3). The most effective antibiotic is ceftazidime (31.1%) that caused inhibition to almost all of the isolated bacterial uropathogens.

Result of ESBL producers

The result of the screening tests for possible ESBL production showed that only 8(18.6) of *Alcaligenes*, 4(19.0) of *Klebsiella pneumoniae* and 1(16.7) of *Myroides* were confirmed phenotypically to be ESBL producers (Table 2) in reference to CLSI Performance Standard for Antimicrobial Susceptibility Testing which shows inhibition zone diameter of ≤ 22 mm with Ceftazidime (30 μ g), ≤ 27 mm with Ceftriaxone (30 μ g) and ≤ 27 mm with Augmentin (30 μ g).

Comparative distribution of the ESBL-producing isolates between the two hospitals

Comparative distribution of the ESBL-producing bacterial isolates between the two hospitals is shown in Figure 1. Of the 13 ESBL-producing isolates, 9 isolates (5 *Alcaligenes*, 3 *Klebsiella pneumoniae* and 1 *Myroides*) are from Dutse General Hospital (DGH), while 4 isolates (3 *Alcaligenes* and 1 *Klebsiella pneumoniae*) are from Rasheed Shekoni Teaching Hospital (RSTH).

Molecular analysis

Gene extraction and amplification

The product of PCR amplicons were separated using gel electrophoresis are presented in Figure 2 and DNA base pair of 750pb

Post PCR phylogenetic analysis

The results for phylogenetic analysis and molecular systematics of the isolates were presented in Figures 3-5 through 5. The evolutionary distance was presented using bootstrapping.

Discussion

This study, observed high prevalence of Gram-negative bacilli 70(94.5%) compared to that of Gram-positive cocci 4(5.4%). Among the bacterial isolates, *Alcaligenes* (58.1%) was the most prevalent uropathogen followed by *Klebsiella pneumoniae* (28.3%), *Myroides* (8.1), *Staphylococcus aureus* (5.4%). This is consistent with the reports of Otajevwo [9] which recorded *Alcaligenes* sp and *Klebsiella* sp as the most prevalent uropathogens. The result differs from reports of Aboderin [10] which recorded *Pseudomonas aeruginosa* and *Klebsiella* sp and that of Oluremi [11] which revealed 85.0% of Gram-negative bacilli of which *E. coli* and *Klebsiella* sp as the most prevalent uropathogens. Although *klebsiella* sp are commonly prevalent in all the study but the variation in other microorganisms isolated further reveals the different species of uropathogens prevalent within the location of study.

The highest incidence was observed among age group of 21-30(27.5%) and the lowest seen in 31-40(4.5%). This could probably be due to the fact that greater number of the subject fall within ages 21-30 which represents sexually and reproductively active age group. The analysis showed that there was no statistical significant difference ($X^2=5.759$ P=0.056) in the prevalence of UTI between the age groups. This study observed that multigravida had an increased risk factor of developing bacteriuria among pregnant women, which could be as a result of their reproductive system becoming more vulnerable to infections after successive pregnancies. Although this differs with that of Ranjan [12] who reported highest incidence in primigravida and lower incidence in multigravida (Table 1). More so, in this study, highest incidence of UTI is seen in second trimester (19.0%) followed by first trimester (10.0%) and least is seen in third trimester (8.0%), the result was in contrast with Ranjan [12] whose highest incidence of UTI

Table 3: Antibiotic susceptibility profile of the isolated uropathogens against the selected antibiotic.

Isolated uropathogens	ANTIBIOTICS USED													
	CAZ (%)		CTX (%)		AUG (%)		TET (%)		CN (%)		OFX (%)		MEAN (%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
GRAMNEGATIVE														
<i>Alcaligene</i>	14	4	9	5	9	3	9	10	4	10	8	10	9	8
n=43(58.9%)	32.6	16.3	20.9	23.3	20.9	7.0	20.9	23.3	9.3	23.3	18.6	23.3	20.9	18.6
<i>K. pneumoniae</i>	5	2	4	2	3	1	4	3	0	3	4	3	3	3
n=21(28.4%)	23.8	19.0	19.0	14.3	14.3	4.7	19.0	14.3	0.0	14.3	19.0	14.3	14.3	14.3
<i>Myroides</i>	3	1	1	1	1	0	1	2	0	2	1	2	1	2
n=6(8.1%)	50.0	33.3	16.7	50.0	16.7	0.0	16.7	33.3	0.0	33.3	16.7	33.3	16.7	33.3
GRAMPOSITIVE														
<i>S. aureus</i>	1	1	0	0	0	1	0	2	0	2	0	2	0	2
n=4(5.4%)	25.0	50.0	0.0	50.0	0.0	25.0	0.0	50	0.0	50.0	0.0	50.0	0.0	50.0
Total	23	8	14	8	13	5	8	17	4	17	13	17	13	15
n=74(100.0%)	31.1	10.8	19.0	10.8	17.6	6.7	10.0	23.0	5.4	23.0	17.6	23.0	17.6	20.3

Where; CAZ: Ceftazidime; CTX: Ceftriaxone; AUG: Augmentin; TET: Tetracycline; CN: Gentamicin; OFX: Ofloxacin; S: Sensitive; R: Resistant

is seen in third trimester (48.0%) followed by second trimester (45.0%). These variations could be a mere occurrence resulting from subjects' exposure level and personal hygiene. The result of this study is not significant at ($p < 0.05$) which means there is no association between gravidity and incidence of UTI in pregnancy. The socio-demographic data analyzed statistically showed no significant relationship with UTI ($P < 0.05$).

The prevalence of UTI was found to be highest in DGH 54(27.0%) than RSTH 20(10.0%). The reason for high prevalence of UTI in DGH could be due to socio economic status and poor genital hygienic practice. Pearson Chi-square analysis showed that the difference in the prevalence of UTI between the two hospitals was statistically significant ($X^2=24.796$, $P < 0.001$, $OR=0.213$).

The prevalence rate of urinary tract infection (UTI) as revealed by this study was 37.0%. This was close to that obtained from similar UTI studies by Ranjan [12] recorded 35% prevalence rate at SVR maternity Hospital Bhimavaram, India while Akinola [13] recorded 40.0% prevalence rate among antenatal patients in University of Ilorin Teaching Hospital and Rekha [14] recorded 30.5% prevalence rate in Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. Similarly, Muhammad [15] recorded lower prevalence rate of 15.8% at Murtala Muhammed Specialist Hospital, Kano and Omonigho [16] who also reported prevalence rates of 22.3%. Much higher prevalence rates have been reported by authors; Okonko [17] recorded prevalence rate of 47.5% at antenatal clinics at Oluyoro Catholic Hospital Ibadan and Kolawole [18] recorded prevalence rate of 60% among outpatients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria while Gayathri [19] recorded prevalence rates of 56% among pregnant women at kanayo specialist and General hospital Onitsha. Of the 74 isolated uropathogens tested against the antibiotics, 9(20.9%) strains of *Alcaligenes*, 3(14.3%) of *Klebsiella pneumoniae* and 2(33.3%) of *Myroides* sp was sensitive to all the selected antibiotics used (Table 3).

Of the 70 isolated Gram-negative bacteria, ESBL production was seen in 13(17.6%) isolates of which 8(18.6%) were *Alcaligenes*, 4(19.0%) were of *Klebsiella* sp and 1(16.7%)

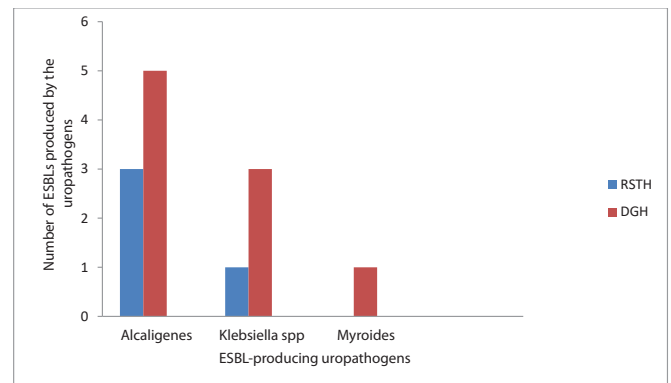


Figure 1: Comparative distribution of ESBL-producing isolates between the two assayed hospitals



Figure 2: Agarose gel electrophoresis indicating the various plasmid bands of isolated bacteria from urine. E: *Alcaligenes* sp; K: *Klebsiella pneumoniae*; Pse: *Myroides* sp

was *Myroides* sp. Comparatively, the prevalence of ESBLs in urinary isolates was higher in *Klebsiella spp* (19.0%) followed by *Alcaligenes* (18.6%) in this study. Prevalence rates of 12.6%, 20.0% and 21.6 % was obtained by Dong [20], Onwuezobe [4], and Motayo [21] respectively. The antibiotic susceptibility testing revealed increasing resistance with Ofloxacin, Gentamicin and Tetracycline that resisted strains of all isolated uropathogens (Table 3). This low sensitivity of these drugs may



Figure 3: Phylogenetic Neighbour joining tree for Isolate *Alcaligenes* sp.

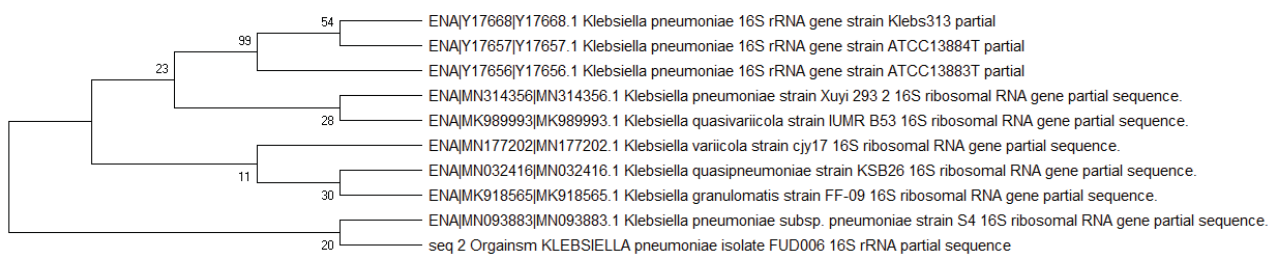


Figure 4: Phylogenetic Neighbour joining tree for Isolate *Klebsiella pneumoniae*.



Figure 5: Phylogenetic Neighbour joining tree for Isolate *Myroides* sp.

be due to development of resistance genes by pathogens as a result of antibiotic abuse or self-medication [21–26].

In this study, the most effective antibiotic is ceftazidime that was sensitive to strains of all isolated uropathogens. The possible reason for the clinical effectiveness of ceftazidime compared to other antibiotics may be due to its limited use and abuse by patients. The molecular enumeration of ESBL-producing uropathogens was observed using 16S rRNA partial sequencing analyses [26–33].

Conclusion

The observed prevalence of UTI among pregnant women in this study was 37.0%. Past history of UTI, contraceptive use and cleaning method after urination or defecation contributed to constitute risk factors for acquiring UTI and the predominant uropathogens are *Alcaligenes*, *Klebsiella* species, *S. aureus* and *Myroides*. The prevalence of ESBL-producing Gram-negative uropathogens was 17.6%.

The ESBL-producing bacteria among UTI pregnant was found to be associated with risk factors such as past histories of exposure to a specific antibiotic, UTI and recent antibiotic use.

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