

## Prevalence of extended-spectrum $\beta$ -lactamase producing isolates from asymptomatic bacteriuria among students in a tertiary institution in Ibadan, Nigeria.

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**Abstract:** Indiscriminate use of antibiotics, particularly the extended-spectrum antibiotics in Nigeria has assisted the principle of selection pressure for the highly resistant strains of bacteria. The aim of this study is to investigate the prevalence of asymptomatic bacteriuria as well as antibiotic susceptibility and extended-spectrum beta-lactamase production among bacterial isolates from healthy students in a tertiary institution in southwest Nigeria. The results of the mid-stream urine catch that was collected and analysed in a total of 100 students, all within the age range 17 to 26 years, were found to contain bacterial isolates of *Staphylococcus aureus* (5%), *Escherichia coli* (3%), *Citrobacter freundii* (4%), *Proteus* spp. (26%), *Klebsiella* spp. (40%), *Morganella morganii* (4%) and *Providencia stuartii* (4%). Visible bacterial growth occurred in 86% of the urine samples with 41% from males and 45% from females. Antibiotics such as penicillin, cephalosporin, quinolone, aminoglycoside, macrolide and nitrofurantoin were used for antibiotic susceptibility test against the isolates. Extended-spectrum beta-lactamase production was determined among cephalosporin resistant Gram-negative isolates. This study showed high prevalence of asymptomatic bacteriuria associated with multidrug resistance and some ESBL-producing bacteria among healthy students of University of Ibadan. Thus concerted effort in the control of the use of extended-spectrum antibiotics should be made, so as to avoid high prevalence of human reservoirs of multidrug resistant and ESBL-producing bacteria. <http://www.sciencepub.net/nature>.

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### Introduction

Asymptomatic bacteriuria is defined as a significant bacterial isolate count greater than or equal to  $10 \times 10^3$  colony-forming units per milliliter ( $\geq 10^5$  cfu/mL) of urine in two consecutive fresh (clean catch) urine specimens (Nicolle, 2003). Significant asymptomatic bacteriuria is a risk factor for symptomatic urinary tract infection and septicemia among predisposed individuals indicating an active infection of the urinary tract (Alebiosu *et al.*, 2003). Asymptomatic bacteriuria occurs in small number of healthy individuals and is common among females particularly, sexually active women (Richard *et al.*, 2006). Its prevalence varies with age, sex, sexual activity, and the presence of genitourinary abnormalities (Nicolle, 2003). In healthy women, the prevalence of bacteriuria increases with age, from about 1% in females of 5 to 14 years of age to more than 20% in women of at least 80 years of age (Richard *et al.*, 2006). Infecting organisms are diverse and include the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Enterococcus* species, *Staphylococcus aureus* and group B *Streptococcus* (Richard *et al.*, 2006; Chukwu *et al.*, 2011). *Escherichia coli* is the most common organism isolated from patients with asymptomatic

bacteriuria (Richard *et al.*, 2006; Dalal *et al.*, 2009). Although certain patients having diabetes, infected kidney stones, kidney transplant, older age and pregnant women are at a higher risk of developing kidney infection if they have asymptomatic bacteriuria, most healthy patients with asymptomatic bacteriuria do not need treatment. However, in this era of abuse and irrational use of antibiotics reported in Nigeria (Tamuno *et al.*, 2011), the possibility of healthy individuals with persistent asymptomatic bacteriuria becoming a reservoir of multidrug resistant and extended-spectrum beta-lactamase producing bacteria was the concern for carrying out this study. Extended-spectrum  $\beta$ -lactamases (ESBLs) are a rapidly evolving group of  $\beta$ -lactamases which also have the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid (Bush *et al.*, 1995; Paterson and Bonomo, 2005). The incidence of extended-spectrum beta-lactamase producing bacteria has been reported in both the community and hospital settings in some parts of the world including Nigeria (Bonnet, 2004; Pitout *et al.*, 2005; Paterson and Bonomo, 2005; Soge *et al.*, 2006; Canton and Coque, 2006; Mendonca *et al.*, 2007; Okesola *et al.*, 2009; Tijjani *et al.*, 2012) and several studies have found a relationship between

the use of third-generation cephalosporins and acquisition of ESBL-producing strains (Ariffin *et al.*, 2000). This study however, investigated the prevalence of multidrug resistance and ESBLs production among bacterial isolates obtained from urine of healthy students of the University of Ibadan, Nigeria.

## Materials and Methods

### Collection of urine samples

One hundred students (50 males and 50 females) between the ages 17 and 26 years, from the University of Ibadan, voluntarily participated in the study. After filling a questionnaire, mid-stream urine clean catch sample was collected aseptically in sterile wide mouth sample bottles and were immediately sent to the laboratory as soon as it was produced by the participating students. The samples were screened for the presence of bacteria within 24 hours of collection. Students currently on antibiotic treatment or that have been treated for urinary tract infection as far as two months before this study were excluded from the research. The participating students were taught how to collect their urine samples without contaminating the samples with their hands and external genital.

### Bacteria Isolation and Identification

A loop-full of urine was streaked on Nutrient agar, Mannitol salt agar, cetrimide agar and MacConkey agar media. The culture plates were incubated at 37°C for 24 hours. Plates that showed growth were observed and the colonies were further characterized by Gram stain and standard biochemical tests. The identified isolates were streaked on agar slants and stored in a refrigerator for later use.

### Antibiotic susceptibility test

The bacterial isolates were subjected to antibiotic susceptibility testing against ten antibiotics belonging to the penicillin, cephalosporin, aminoglycoside, quinolone, macrolide and nitrofurans classes using the disc diffusion method on Mueller Hinton agar according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2011). The antibiotics included: amoxicillin-clavulanic acid (20/10 µg), cefuroxime (30 µg), cefixime (5 µg), ceftazidime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), erythromycin (5 µg) and nitrofurantoin (300 µg).

### Detection of Extended-Spectrum Beta-Lactamase (ESBL) Production

ESBL production was determined among the Gram negative bacteria resistant to third generation cephalosporins by double-disc synergy test with

ceftazidime (30 µg) and cefotaxime (30 µg) discs placed 20mm centre-to-centre around amoxicillin-clavulanic acid (20/10 µg) disc. This was done on the surface of Mueller Hinton agar plate inoculated with the isolates suspension equivalent to 0.5 McFarland standard by surface spreading using sterile swab sticks. After incubation at 37°C for 24 hours, ESBL production was inferred when the zone of inhibition around the cephalosporin discs was expanded by ≥5mm by the presence of clavulanic acid.

## Results

Of the 100 urine samples collected (50 males and 50 females), 86 (41 from male, 45 from female) gave visible bacterial growth after 24 hours of incubation. Table 1 shows the various bacteria isolated from the urine samples. *Klebsiella* spp. (46.5%) were found to be the most common bacteria isolated followed by *Proteus* spp. (30.2%) with *Klebsiella* more common in females (65%) than in males (35%) while *Proteus* spp was more common in males (65.4%) than females (34.6%). However, *E. coli* was found in female only. The antibiotic susceptibility test revealed that 96.5% of the isolates were resistant to amoxicillin-clavulanic acid, 95.4% to cefixime, 89.5% to cefuroxime, 80.2% to ceftazidime and 76.7% to ceftriaxone. Resistance to ciprofloxacin and ofloxacin was 33.7% and 20.9% respectively. Gentamicin had 41.9%, erythromycin 89.5% and nitrofurantoin 76.7% resistance against the isolates. *S. aureus*, *Klebsiella* spp., *M. morgannii*, *P. stuartii* and *C. freundii* isolates all had 100% resistance to amoxicillin-clavulanic acid, *E. coli* and *P. stuartii* had 100% resistance to ceftazidime, cefixime and cefuroxime while *M. morgannii*, *P. stuartii* and *C. freundii* were susceptible to ofloxacin (100%). *S. aureus* had 100% susceptibility to nitrofurantoin, ciprofloxacin and gentamicin (table 2). Among the 86 isolates, ESBL-production was detected in 30 (34.9%) of which 19 (22.1%) were from males and 11 (12.8%) from females. They included: *Providencia stuartii* (1.2%), *Moganella morganni* (3.5%), *Klebsiella* spp (15.1%), *Citrobacter freundii* (3.5%) and *Proteus* spp (11.6%). *C. freundii* and *M. morganni* gave the highest percentage ESBL-production (75%) compared to *Proteus* spp. (38.5%) and *Klebsiella* spp. (32.5%) as presented in table 3. Prevalence of multidrug resistance among the 86 isolates was 81.4% which was highest in *P. stuartii* (100%), followed by *Klebsiella* spp (87.5%), *Proteus* spp (84.6%), *M. morganni* (75%), *C. freundii* (75%) and *E. coli* (66.7%).

Table 1: Occurrence of bacterial isolates among male and female students

Bacteria	Total Number/ Percentage of isolates	Number /Percentage of isolates by sex	
		Male	Female
<i>Staphylococcus aureus</i>	5 (5.8%)	3 (60%)	2 (40%)
<i>Escherichia coli</i>	3 (3.5%)	0 (0%)	3 (100%)
<i>Proteus spp.</i>	26 (30.2%)	17 (65.4%)	9 (34.6%)
<i>Klebsiella spp.</i>	40 (46.5%)	14 (35%)	26 (65%)
<i>Moganella morganni</i>	4 (4.7%)	2 (50%)	2 (50%)
<i>Providencia stuartii</i>	4 (4.7%)	1 (25%)	3 (75%)
<i>Citrobacter freundii</i>	4 (4.7%)	4 (100%)	0 (0%)
Total number of isolates	86 (100%)	41 (47.7%)	45 (52.3%)

Table 2: Number and percentage susceptibility pattern of the isolates to selected antibiotics

Antibiotics	<i>S. aureus</i> n = 5	<i>E. coli</i> n = 3	<i>Proteus spp.</i> n = 26	<i>Klebsiella spp.</i> n = 40	<i>M. morgannii</i> n = 4	<i>P. stuartii</i> n = 4	<i>C. freundii</i> n = 4
AMC	5(100%)	1(33.3%)	25(96.2%)	40(100%)	4(100%)	4(100%)	4(100%)
CRX	3 (60%)	3 (100%)	23(88.5%)	38(95%)	3(75%)	4(100%)	3(75%)
CXM	5(100%)	3(100%)	24(92.3%)	39(97.5%)	4(100%)	4(100%)	3(75%)
CAZ	2 (40%)	3(100%)	22(84.6%)	33(82.5%)	3(75%)	4(100%)	2(50%)
CTR	2 (40%)	2(66.7%)	22(84.6%)	32(80%)	2(50%)	4(100%)	2(50%)
GEN	0 (0%)	2(66.7%)	12(46.2%)	17(42.5%)	2(50%)	2(50%)	1(25%)
CPR	0 (0%)	1(33.3%)	9(34.6%)	14(35%)	3(75%)	1(25%)	1(25%)
OFL	1(20%)	1(33.3%)	7(26.9%)	9(22.5%)	0(0%)	0(0%)	0(0%)
ERY	1 (20%)	3(100%)	23(88.5%)	38(95%)	4(100%)	4(100%)	4(100%)
NIT	0 (0%)	1(33.3%)	21(80.8%)	35(87.5%)	3(75%)	4(100%)	2(50%)

**Key:** AMC-amoxicillin-clavulanic acid (20/10 µg), CRX-cefuroxime (30 µg), CXM-cefixime (5 µg), CAZ-ceftazidime (30 µg), CTR-ceftriaxone (30 µg), GEN-gentamicin (10 µg), CPR-ciprofloxacin (5 µg), OFL-ofloxacin (5 µg), ERY-erythromycin (5 µg) and NIT-nitrofurantoin (300 µg).

Table 3: Distribution of Multidrug resistant and ESBL-producers among the isolates

Bacteria isolates	MDR isolates			ESBL-producers		
	Total	Male	Female	Total	Male	Female
<i>Staphylococcus aureus</i> (n = 5)	1 (20%)	1 (20%)	0 (0%)	ND	ND	ND
<i>Escherichia coli</i> (n = 3)	2 (66.7%)	0 (0%)	2 (66.7%)	0 (0%)	0 (0%)	0 (0%)
<i>Proteus spp.</i> (n = 26)	22 (84.6%)	13 (50%)	9 (34.6%)	10 (38.5%)	7 (26.9%)	3 (11.5%)
<i>Klebsiella spp.</i> (n = 40)	35 (87.5%)	10 (25%)	25 (62.5%)	13 (32.5%)	6 (15%)	7 (17.5%)
<i>Moganella morganni</i> (n = 4)	3 (75%)	1 (25%)	2 (50%)	3 (75%)	2 (50%)	1 (25%)
<i>Providencia stuartii</i> (n = 4)	4 (100%)	1 (25%)	3 (75%)	1 (25%)	1 (25%)	0 (0%)
<i>Citrobacter freundii</i> (n = 4)	3 (75%)	3 (75%)	0 (0%)	3 (75%)	3 (75%)	0 (0%)
Total number (n = 86)	70 (81.4%)	29 (33.7%)	41 (47.7%)	30 (34.9%)	19 (22.1%)	11 (12.8%)

**Key:** MDR-Multidrug resistant; ESBL-Extended-Spectrum Beta-Lactamase; ND-Not determined

## Discussion

Although, asymptomatic bacteriuria in a healthy individual does not require treatment and may disappear within a few days but the possibility of having healthy individuals as carriers of multidrug resistant and ESBL-producing bacteria in their urine is inevitable. Several studies in Nigeria have shown that abuse and indiscriminate use of antibiotics by people practicing self-medication are partly

responsible for the high prevalence of multidrug resistance and ESBL-producing bacteria in both community and hospital acquired infections (Yah *et al.*, 2008; Tamuno *et al.*, 2011). People tend to treat minor infections or certain ailments that may not require antibiotics with broad-spectrum or extended-spectrum antibiotics thereby aiding the principle of selection pressure for the highly resistant strains in such individuals. This study recorded high prevalence

of asymptomatic bacteriuria among the students of University of Ibadan in southwest Nigeria with high level of multidrug resistant and moderate level of ESBL-producing bacteria. It is interesting to note that all the volunteers in this study have at one time or the other taken antibiotics without prescription for several ailments such as cough, sour-throat, urinary tract infections, typhoid fever, otitis media, boil and others. We suggest therefore that abuse and indiscriminate use of antibiotics among the students of the institution were crucial factors responsible for the high level of the multidrug resistance observed in this study as revealed from the questionnaires. All the volunteers have at one time taking antibiotics.

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