

PHENOTYPIC EXPRESSION OF EXTENDED SPECTRUM BETA-LACTAMASES AND ANTIBIOGRAM OF URO-PATHOGENIC BACTERIAL ISOLATES FROM OUT-PATIENTS ATTENDING SOME PRIVATE HOSPITALS IN UYO, NIGERIA

¹Etukudo Idongesit Udofot, ¹Onyeagba Reginald Azuonye, ²Akinjogunla Olajide Joseph, ¹Ibe Chibuike and ³Ikpe Michael Emmanuel

¹Department of Microbiology, Faculty of Biological Sciences, Abia State University, Uturu, Abia State, Nigeria.

²Department of Microbiology, Faculty of Sciences, University of Uyo, P.M.B.1017, Uyo, Akwa Ibom State, Nigeria.

³Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

*Corresponding Author's Email Address: idyetukudo@gmail.com

Phone: +2348031345533

ABSTRACT

Urinary tract infection is a common health problem in both community and nosocomial setting. Microbiological analysis of mid-stream urine (MSU) of out-patients were carried out using standard microbiological technique. The presence of glucose, protein, ketone, leucocyte, bilirubin and nitrite were found out using dip sticks. The phenotypic detection of extended spectrum beta- lactamase (ES β L) and antibiogram of isolates were determined by disc diffusion method. Of the 150 MSU samples from out-patients, 34.7% had significant bacteriuria (SBU), while 65.3% showed no significant bacteriuria. There was no statistically significant relationship between the occurrences of SBU among subjects based on ages ($p=0.567$), marital status ($p=0.063$), educational levels ($p=0.789$) and occupation ($p=0.134$) whereas based on gender, there was statistically significant difference at $p < 0.05$. Sixty (40.0%) of MSU samples had leucocytes, 29.3% contained nitrite, 27.3% contained urobilinogen, 28.7% contained protein, 16.0% had ketone and bilirubin each, 20.7% had glucose, while 12.7% and 9.3% had yeast cells and cellular cells, respectively. Bacterial genera isolated were *Staphylococcus*, *Escherichia*, *Pseudomonas*, *Streptococcus*, *Klebsiella*, *Serratia*, *Enterococcus* and *Enterobacter*. *Staphylococcus aureus*, *Streptococcus* spp., *E. coli*, coagulase negative *Staphylococcus* spp and *P. aeruginosa* were highly sensitive to Ciprofloxacin and Gentamycin, while *S. marcescens* and *Enterobacter* spp were moderately resistant to Reflacine and Augmentin. ES β Ls were detected in *E. coli* (43.5%), *K. pneumoniae* (58.8%), *P. aeruginosa* (63.0%), *S. marcescens* (60.0%) and *Enterobacter* species (50.0%). This study has revealed the necessity to routinely carry out medical examinations of subjects attending various hospitals for asymptomatic bacteriuria so as to reduce the prevalence of SBU and prevent symptomatic infection and its complications.

Keywords: Bacteriuria, Antibiotic, Dipstick, Microscopic, Susceptibility, Uyo

INTRODUCTION

Urinary tract infection (UTI) is one of the most common causes of hospitalization and health problems in both community and nosocomial setting (Stamm and Hooton, 1993). The UTI is, either

symptomatic or asymptomatic infection, caused by pathogenic microorganisms that invade the entire tract or confined to either lower tract or upper tract resulting in urethritis, prostatic and pyelonephritis (Tigist *et al.*, 2016). The clinical manifestations of UTI depend on the portion of the urinary tract involved, causative organisms, severity of the infection, and patient's ability to mount an immune response to it (Akinjogunla *et al.*, 2010). The frequency of UTIs depends on many risk factors such as diabetes mellitus, age, immune-suppression and neurological disorders (Redder *et al.*, 2016) and urethral catheterization (Tosin *et al.*, 2018). The UTI is frequently caused by bacteria, predominantly *Escherichia coli* and other bacterial isolates such as *Klebsiella pneumoniae*, *Proteus* sp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus* sp and coagulase negative (CoN) *Staphylococcus* sp (Akinjogunla and Divine-Anthony, 2013; Syed *et al.*, 2019). The relative frequency of these pathogens varies, depending upon age, sex, catheterization, and hospitalization. Asymptomatic bacteriuria is a significant bacterial count ($\geq 10^5$ CFU / ml) of midstream urine sample in an individual without any apparent symptoms of UTI (Smith, 1994; Akinjogunla and Divine-Anthony, 2013). The direct microscopy and chemical analysis of MSU for pH, protein, glucose, ketones, blood, bilirubin, nitrite and other useful parameters also assist the clinicians in the diagnosis of metabolic and systemic disorders (Brauner *et al.*, 1993; Akinjogunla and Divine-Anthony, 2013).

The occurrence of antimicrobial resistance among urinary pathogens has increased globally and the resistance rates to the most commonly prescribed drugs used in the treatment of UTI vary considerably in different areas world-wide (Akinjogunla *et al.*, 2011). Extended-spectrum β -lactamases (ES β Ls) producing bacterial isolates have spread worldwide and have become endemic in several countries (Akinjogunla *et al.*, 2011; Osthoff *et al.*, 2015). The ES β Ls are frequently plasmid mediated and derived from mutations in the classic Temoria (TEM) and Sulphydryl Variable (SHV) genes by amino acid substitution around the active sites (Akinjogunla *et al.*, 2011). This study evaluated the microscopic and chemical analysis of MSU samples, determined the antibiotic susceptibility profiles and ES β Ls production of bacteria isolated.

MATERIALS AND METHODS

Study Area

This study was carried out in Uyo with an estimated population of 451,128 (FRNOG, 2007). Uyo is the capital of Akwa Ibom State and is located between latitudes 5° 02' 37" North and longitudes 7° 54' 06" East (FRNOG, 2007). Uyo has numerous private, secondary and tertiary hospitals.

Collection of Mid-stream Urine

One hundred and fifty (150) Mid-stream urine (MSU) samples were aseptically collected from out-patients, aged ≤ 20 yrs and ≥ 60 yrs, attending some private hospitals within Uyo Metropolis, Akwa Ibom State. The MSU samples were collected from out-patients who had not received antibiotics for the previous five days using sterile containers and transported to microbiology laboratory for microbiological analysis within 1-4 h of collection. Questionnaires reflecting the age, sex, marital status, educational level and occupation were administered to the participants after obtaining their informed verbal consent.

Dipstick Analysis of Urine Samples

The presence of leucocytes, nitrite, urobilinogen, proteins, blood, ketone, bilirubin, glucose, pH and specific gravity in the MSU samples of out-patients were determined using commercially available Urine Analysis Test Strips (Uric 10 CF, ACCU-ANSWER).

Microscopic Examination of Mid-stream Urine Samples

Ten milliliter (10 ml) of MSU sample of each out-patient was aseptically transferred into sterile centrifuge tube and then centrifuged at 3000 rpm for 15 mins. The supernatant was carefully discarded and urine sediment was placed on a clean slide and covered with a clean cover slip. The urine sediment was examined microscopically for presence of pus cells, epithelial cells, yeast cells and crystals using 10 X and 40 X objectives of a light microscope.

Bacteriological Analysis of Mid-stream Urine Samples

One milliliter (1.0 mL) of aliquot from each tenfold-serially diluted MSU sample was aseptically pour-plated into each plate of Cysteine Lactose Electrolyte Deficient (Oxoid, UK). The plates were allowed to solidify, aerobically incubated for 24hrs at 37°C. Then the culture colonies were observed and counted using Digital colony counter (Model: LT-37). Colony counts yielding bacterial growth of $\geq 10^5$ per mL were regarded as significant bacteriuria (SBU) (Ibadin *et al.*, 2006). Pure isolates were obtained by sub-culturing the colonies onto freshly prepared nutrient agar plates and aerobically incubated 24hrs at 37°C. The bacterial isolates were characterized and identified using Gram staining reaction, biochemical tests such as catalase, urease, citrate utilization, oxidase, coagulase, methyl red and sugar fermentation test (Cheesbrough, 2006).

Antibiotic Susceptibility Testing

In vitro antibiotic susceptibility of bacterial isolates was determined by Kirby Bauer disc diffusion technique (CLSI, 2015). Each overnight bacterial suspension was adjusted to 0.5 McFarland turbidity standards. Ten (10) microlitre of each bacterial suspension was aseptically inoculated onto each Mueller-Hinton Agar (MHA) plate. Gram positive disc containing

the following antibiotics: Penicillin (PEN, 10 μ g) ; Ceftazidime (CAZ, 30 μ g); Streptomycin (STP, 30 μ g) ; Ciprofloxacin (CPF, 5 μ g) ; Gentamycin (GEN, 10 μ g) ; Ofloxacin (OFL, 5 μ g) ; Ceftriaxone (CEF, 30 μ g) and Cotrimoxazole (COT, 30 μ g), while the Gram negative disc were : Ofloxacin (OFL, 5 μ g) ; Reflacin (PEX, 5 μ g) ; Augmentin (AU, 30 μ g) ; Ciprofloxacin (CPX, 5 μ g); Cotrimoxazole (SXT, 25 μ g); Gentamycin (GEN, 10 μ g) ; Cephalothin (CEP, 30 μ g) ; Streptomycin (S, 10 μ g) ; Nalidixic Acid (NA, 5 μ g) and Ampicillin (PN, 10 μ g) (Oxoid, UK), were aseptically placed on the surfaces of the MHA plates using sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37°C for 18 h, the zones of inhibition after incubation were observed and measured in millimeters (mm) using a graduated meter rule. The interpretation of measurement as sensitive and resistant was made according to interpretative manual by CLSI (2015).

Detection of Extended Spectrum β lactamase (ES β L) Producing Bacterial Isolates

The ES β L production was confirmed by the Double Disc Synergy Test method. Each overnight bacterial suspension was adjusted to 0.5 McFarland turbidity standards. Ten (10) microlitre of each bacterial suspension was aseptically inoculated onto each MHA plate. A combination disc of amoxicillin-clavulanic acid (AMC; 20/10 μ g) was placed at the center of the MHA plate and cefotaxime (CTX, 30 μ g) and ceftazidime (CAZ, 30 μ g) were placed on either side of the central disc (AMC, 20/10 μ g) at a distance of 20 mm. The plates were incubated for 18 h at 37°C and after incubation, a ≥ 5 mm increase in zones of inhibition for either CAZ and/or CTX tested in combination with AMC confirmed ES β L production (Akinjogunla *et al.*, 2011).

Statistical Analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0) was used for data analysis.

The significant difference in the socio-demographic characteristics among the out-patients at $p \leq 0.05$ were determined using chi-square (χ^2) test.

RESULTS

Of the 150 MSU samples from the out-patients, 34.7% had significant bacteriuria (SBU), while 65.3% showed no significant bacteriuria (Table 1). Age group ≤ 20 yrs had SBU of 19.0%, while age group ≥ 61 yrs had 29.6%. The occurrences of SBU in out-patients based on marital status were: singles (46.2%), married (26.2%), divorced (20.0%) and widowed (23.5%). The occurrences of SBU in out-patients based on educational levels were primary school certificate holders 5/18(27.8%), secondary school certificate holder 13/38 (34.2 %) and tertiary school holder 34/94 (36.2%). The highest occurrence of SBU was found among students with 44.7%, while the lowest occurrence of SBU was among public servants with 21.4%. There was no statistically significant relationship between the occurrences of bacteriuria among the subjects in relation to age ($p=0.567$), marital status ($p=0.063$), educational levels ($p=0.789$) and occupation ($p=0.134$) but in relation to gender, there was statistically significant difference ($p=0.004$) (Table 1).

The percentage occurrence of bacterial isolates from MSU showed that 41.8% of total isolates (225) obtained were Gram positive bacterial isolates, while 58.2% were Gram

negative bacteria. The frequency of occurrences of the nine (9) different bacterial isolates obtained from MSU showed that *Escherichia coli* had the highest prevalence of 28.0%, while *Enterococcus faecalis* had the lowest prevalence of 4.0%. *Klebsiella pneumoniae* was 8.0%, *Streptococcus* sp 9.0%, coagulase negative (CoN) *Staphylococcus* sp 11.0%, *Staphylococcus aureus* 18.0%, *Serratia marcescens* 4.0%, *Pseudomonas aeruginosa* 14.0% and *Enterobacter* sp 4.0% (Fig. 1).

Of the 150 MSU samples collected 10.0%, 19.3%, 14.7%, 6.7% and 12.7% contained crystals, epithelial cells, pus cells, red blood cells (RBCs) and yeast cells, respectively (Table 2). Of the 29 MSU with epithelial cell, age group 21-30 yrs had the highest number of 39.3%, followed by age groups 31-40 yrs with 22.7%, while age groups ≥ 61 yrs had the lowest (3.7%). The highest occurrence of pus cells, yeast cells, and granular cast was obtained among age group 21-30 yrs, while the lowest number of cellular cells and yeast cells was obtained among age group ≥ 61 yrs. The highest percentage (14.3%) of RBCs was obtained among the age group 21-30 yrs, while the lowest percentage (3.7%) of RBCs was obtained among the age group 41-50 yrs (Table 2).

Sixty (40.0%) of MSU samples had leucocytes, 29.3% had nitrite, 27.3% had urobilinogen, 28.7% had protein, 16.7% had blood, 16.0% had ketone, 16.0% had bilirubin and 20.7% had glucose (Table 3). The highest occurrence of leucocytes 50.0% and nitrite 40.0% was obtained in age group 31-40yrs, while the highest occurrence of urobilinogen 53.6%, ketone 55.6% and bilirubin 44.4% was obtained in age group 41-50yrs. The lowest occurrence of leucocytes, nitrite, and ketone was obtained among the age group 21-30yrs. Of 15(25.0%) subjects with glucose in their MSU samples, age group of ≥ 61 yrs had the highest occurrence 4(36.4%), followed by age groups 41-50yrs, 51-60yrs and 31-40yrs with 3(33.0%), 3(27.0%) and 2(20.0%), respectively (Table 3).

Of the 140 Gram négative bacterial isolates obtained, between 61.4 % and 72.9 % were sensitive to Streptomycin, Gentamycin and Ofloxacin, while ≤ 58.6 % showed sensitivity to Pefloxacin and Augmentin. *Escherichia coli* was highly resistant to Penicillin and Augmentin (≥ 45.2 %); *S. marcescens*, *Enterobacter* sp and *E. faecalis* were highly resistant (≥ 55.6 %) to Nalidixic Acid and Cotrimoxazole (Table 4). Of the 85 Gram positive bacterial isolates obtained, ≤ 77.6 % were Ciprofloxacin and Levofloxacin sensitive; 60.0 % isolates were Norfloxacin and Amoxicillin sensitive, while ≥ 58.9 % showed sensitivity to Rifampicin and Erythromycin. *Staphylococcus aureus* was most resistant to Ampicloxacin (51.2%) and least resistant to Levofloxacin (57.1%), *Streptococcus* sp was most resistant to Rifampicin (50.0%) and least resistant to Levofloxacin (25.0%), while CoN *Staphylococcus* sp was most resistant to Rifampicin (47.1%) and least resistant to Levofloxacin (22.4%) (Table 5). The varied antibiotic resistance patterns of Gram negative and Gram positive bacterial isolates from MSU samples are presented in Tables 6 and 7. Of the 131 Gram negative bacterial isolates obtained from the MSU, 51.9% were ES β L producers, while 48.1% were non ES β Ls producers. ES β Ls were detected in *E. coli* 27/62(43.5%), *K. pneumoniae* 10/17(58.8%), *P. aeruginosa* 20/32(63.0%), *S. marcescens* 6/10(60.0%) and *Enterobacter* sp 5/10(50.0%). The

occurrence of the ES β L producing bacterial isolates from MSU samples was not statistically significant from the non-ES β L producers ($p = 0.4371$, $X^2 = 3.776$, $df = 4$) (Table 8)

Table 1: Demographic Characteristics of Out-patients with Significant Bacteriuria in Uyo

Parameters	No of Subjects	No. Positive for Significant Bacteriuria	% Positive for Significant Bacteriuria	χ^2	p-values
Age (yrs)					
≤ 20	21	4	19.0	3.88	0.567
21-30	28	12	42.9		
31-40	22	8	36.4		
41-50	27	11	40.7		
51-60	25	9	36.0		
≥ 61	27	8	29.6		
Gender					
Male	70	16	22.9	8.08	0.004*
Female	80	36	46.0		
Marital Status					
Single	67	31	46.2	7.31	0.063
Married	61	16	26.2		
Divorced	5	1	20.0		
Widowed	17	4	23.5		
Educational Level					
Primary School	18	5	27.8	0.474	0.789
Secondary School	38	13	34.2		
Tertiary	94	34	36.2		
Occupation					
Farmers	4	1	25.0	1.42	0.134
Public Servant	14	3	21.4		
Traders	22	5	22.7		
Civil Servants	34	9	26.5		
Students	76	34	44.7		

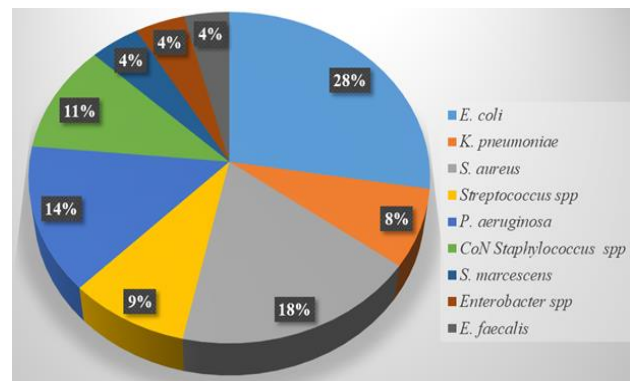


Fig 1: Percentage Occurrence of Bacterial Isolates from Mid-stream Urine Samples of Out-patients

Table 2: Microscopy of Mid-stream Urine Samples in relation to ages of Out-patients

Age(yrs)	No of Samples	Epithelial cell No (%)	Pus cell No (%)	Yeast cell No (%)	Crystals No (%)	Granular Cast No (%)	Cellular Cast No (%)	RBC No (%)
≤ 20	21	4(19.0)	3(14.3)	4(19.0)	2(9.5)	0(0.0)	2(9.5)	2(9.5)
21-30	28	11(39.3)	7(25.0)	7(25.0)	5(17.9)	3(10.7)	4(14.3)	4(14.3)
31-40	22	5(22.7)	3(13.6)	3(13.6)	5(22.7)	2(9.0)	3(13.6)	3(13.6)
41-50	27	4(14.8)	5(18.5)	2(7.4)	2(7.4)	1(3.7)	2(7.4)	1(3.7)
51-60	25	4(16.0)	2(8.0)	2(8.0)	1(4.0)	1(4.0)	2(8.0)	0(0.0)
≥ 61	27	1(3.7)	2(7.4)	1(3.7)	0(0.0)	2(7.4)	1(3.7)	0(0.0)
Total	150	29(19.3)	22(14.7)	19(12.7)	15(10.0)	7(4.7)	14(9.3)	10(6.7)

Key: RBC: Red Blood Cell

Table 3: Dipstick Analysis of Mid-stream Urine Samples of Out-patients

Age(yrs)	No of Samples	Leu No (%)	Nit No (%)	Uro No (%)	Pro No (%)	Bld No (%)	Ket No (%)	Bil No (%)	Glu No (%)
≤ 20	21	7(33.3)	3(14.3)	2(9.5)	3(14.3)	0(0.0)	2(9.5)	3(14.3)	2(9.5)
21-30	28	8(28.6)	7(25.0)	6(21.4)	4(14.3)	2(7.1)	2(7.1)	2(7.1)	5(7.1)
31-40	22	14(63.6)	11(50.0)	9(40.9)	8(36.4)	2(9.1)	2(9.1)	3(13.6)	2(9.1)
41-50	27	13(48.1)	10(37.0)	12(44.4)	8(29.6)	4(14.8)	8(29.6)	8(29.6)	5(18.5)
51-60	25	11(44.0)	7(28.0)	5(20.0)	13(52.0)	9(36.0)	6(24.0)	4(16.0)	6(24.0)
≥ 61	27	7(25.9)	6(22.2)	7(25.9)	7(25.9)	8(29.6)	4(14.8)	4(14.8)	11(40.7)
Total	150	60(40.0)	44(29.3)	41(27.3)	43(28.7)	25(16.7)	24(16.0)	24(16.0)	31(20.7)

Keys: Leu: Leucocytes; Nit: Nitrite; Uro: Urobilinogen; Pro: Protein; Bld: Blood; Ket: Ketone; Bil: Bilirubin; Glu: Glucose

Table 4: Antibiotic Sensitivity of Gram Negative Bacterial Isolates from Mid-stream Urine Samples of Out-patients

Bacterial Isolates	No of Isolates	OFX No (%)	PEF No (%)	CPX No (%)	AU No (%)	CN No (%)	S No (%)	CEP No (%)	NA No (%)	SXT No (%)	PN No (%)
<i>E. coli</i>	62	41(66.1)	35(56.5)	48(77.4)	34(54.8)	45(72.6)	41(66.1)	42(67.7)	45(72.6)	37(59.7)	30(48.4)
<i>K. pneumoniae</i>	17	12(70.6)	10(58.8)	13(76.5)	14(82.4)	13(76.5)	11(64.7)	11(64.7)	10(58.8)	12(70.6)	11(64.7)
<i>P. aeruginosa</i>	32	25(78.1)	19(59.4)	23(71.9)	19(59.4)	25(78.1)	21(65.6)	23(71.9)	19(59.4)	19(59.4)	17(53.1)
<i>S. marcescens</i>	10	6(60.0)	4(40.0)	6(60.0)	5(50.0)	7(70.0)	5(50.0)	6(60.0)	4(40.0)	4(40.0)	4(40.0)
<i>Enterobacter spp</i>	10	7(70.0)	5(50.0)	5(50.0)	4(40.0)	6(60.0)	5(50.0)	5(50.0)	4(40.0)	4(40.0)	3(30.0)
<i>E. faecalis</i>	9	6(66.7)	6(66.7)	5(55.6)	6(66.7)	6(66.7)	7(77.8)	4(44.4)	4(44.4)	3(33.3)	3(33.3)
Total	140	97(69.3)	79(56.4)	100(71.4)	82(58.6)	102(72.9)	90(64.3)	90(64.3)	86(61.4)	79(56.4)	68(48.6)

Keys: OFX: Ofloxacin; PEX: Reflacine; AU: Augmentin; CPX: Ciprofloxacin; CN: Gentamycin; S: Streptomycin; CEP: Cephalothin; NA: Nalidixic Acid; SXT: Cotrimoxazole; PN: Ampicillin

Table 5: Antibiotic Sensitivity of Gram Positive Bacterial Isolates from Mid-stream Urine Samples of Out-patients

Bacterial Isolates	No of Isolates	CPX No (%)	NB No (%)	CN No (%)	AML No (%)	S No (%)	RD No (%)	E No (%)	CH No (%)	APX No (%)	LEV No (%)
<i>S. aureus</i>	41	29(70.7)	26(63.4)	30(73.2)	25(61.0)	23(56.1)	22(53.7)	22(53.7)	21(51.2)	20(48.8)	32(78.1)
<i>Streptococcus sp</i>	20	15(75.0)	12(60.0)	14(70.0)	13(65.0)	12(60.0)	10(50.0)	11(55.0)	12(60.0)	12(60.0)	15(75.0)
CoN- <i>Staphylococcus sp</i>	24	17(70.8)	13(54.2)	16(66.6)	13(54.2)	15(62.5)	13(54.2)	15(62.5)	14(58.3)	15(62.5)	19(79.2)
Total	85	61(71.8)	51(60.0)	60(70.6)	51(60.0)	50(58.9)	45(52.9)	48(56.5)	47(55.3)	47(55.3)	66(77.6)

Keys: CPX: Ciprofloxacin; NB: Norfloxacin; CN: Gentamycin; AML: Amoxicillin; S: Streptomycin; RD: Rifampicin; E: Erythromycin; CH: Chloramphenicol; APX: Ampicloxacin; LEV: Levofloxacin; CoN: Coagulase negative

Table 6: Antibiotic Resistance Pattern of Bacterial Isolates from MSU of Out-patients

Bacterial Isolates	Resistance Pattern	No (%) of Occurrences
<i>E. coli</i>	OFX-PEF-AU-CN-CEP-NA-SXT-PN	4(6.45)
	SXT	9(14.5)
	PN-CN-NA	1(1.61)
	SXT-PN	8(12.9)
	OFX-SXT	4(6.45)
	S-CEP	3(4.84)
	OFX-PEF-AU-CN-CEP-NA-PN	4(6.45)
	PEF-CPX-AU-CN-S	2(3.22)
	OFX-PEF-CPX-AU-CN-CEP-PN	1(1.61)
	OFX-PEF-CPX-AU-CN-CEP	3(4.84)
	OFX-PEF-AU-CN-S-CEP-PN	5(8.06)
	PEF-CPX-AU-S-NA-PN	9(14.5)
	<i>K. pneumoniae</i>	OFX-S-SXT
CEP-NA-PN		3(17.6)
PEF		2(11.8)
OFX-PEF-CPX-AU-CN-S-NA-SXT		3(17.6)
CEP-PN		3(17.6)
PEF-CN-NA		1(5.88)
PEF-CPX-S-CEP		1(5.88)
<i>S. marcescens</i>	OFX-PEF-AU-S-CEP-NA-SXT-PN	4(40.0)
	PEF-CPX-CN-NA-SXT-PN	2(20.0)
	CPX-AU-CN-S	1(10.0)
	CPX	1(10.0)
<i>Enterobacter</i> sp	OFX-PEF-CPX-AU-S-CEP-NA-SXT-PN	3(30.0)
	CN-PN	2(20.0)
	AU-CN-S-CEP-NA-SXT-PN	2(20.0)
	PEF-CPX	1(10.0)
	PEF-CPX-AU-CN-NA	1(10.0)
<i>P. aeruginosa</i>	CEP-NA	5(15.6)
	SXT-PN	3(9.38)
	NA	2(6.25)
	OFX	2(6.25)
	OFX-PEF-CPX-AU-CN-S-SXT-PN	5(15.6)
	PEF-CPX-AU-S-NA-PN	4(12.5)
	PEF-AU-CN-S-CEP-SXT-PN	2(6.25)
	CEP-NA-SXT	1(3.13)
	PEF-AU-CEP-NA-SXT-PN	1(3.13)
	PEF-AU-SXT	1(3.13)

Keys: OFX: Ofloxacin; PEX: Reflacine; AU: Augmentin; CPX: Ciprofloxacin; CN: Gentamycin; S: Streptomycin; CEP: Cephalothin; NA: Nalidixic Acid; SXT: Cotrimoxazole; PN: Ampicillin

Table 7: Antibiotic Resistance Pattern of Bacterial Isolates from MSU of Out-patients

Bacterial Isolates	Resistance Pattern	No (%) of Occurrences
<i>E. faecalis</i>	OFX	1(11.1)
	OFX-PN	1(11.1)
	AU-CN-CEP-NA-SXT	1(11.1)
	OFX-PEF-SXT-PN	1(11.1)
	CPX-S-CEP-NA-SXT-PN	2(22.2)
	PEF-CPX-AU-CN-CEP-NA-SXT-PN	2(22.2)
<i>S. aureus</i>	CPX-NB-CN-AML-E-CH-APX-LEV	6(14.6)
	APX	1(2.44)
	CPX-CN-AML-RD-CH-APX	2(4.88)
	S-RD	5(12.2)
	NB	4(9.76)
	AML-NB-S-E-LEV	1(2.44)
	S-RD-E-CH-APX-LEV	2(4.88)
	S-RD-E-CH-APX	3(7.32)
	CPX-NB	4(9.76)
	AML-S-RD-E-CH-APX	4(9.76)
	CN-AML-S-RD-E-CH-APX	3(7.32)
	<i>Streptococcus sp</i>	CPX-NB-S-RD-CH-APX-LEV
CN-AML-S-RD-E-APX		3(15.0)
NB-AML		2(10.0)
E-CH		4(20.0)
CPX		1(5.0)
CN-S-RD-E		1(5.0)
APX-LEV		1(5.0)
NB-CN-AML-RD		2(10.0)
CoN- <i>Staphylococcus sp</i>	CPX-NB-CN-S-E-CH-LEV	5(20.8)
	AML-RD-CH-APX	5(20.8)
	NB-AML	3(12.5)
	RD	4(16.7)
	CPX-S-RD-E	1(4.17)
	NB-CN-AML-S-E-APX	3(12.5)
	CPX-RD-APX	1(4.17)

Keys: CPX: Ciprofloxacin; NB: Norfloxacin; CN: Gentamycin; AML: Amoxicillin; S: Streptomycin; RD: Rifampicin; E: Erythromycin; CH: Chloramphenicol; APX: Ampicloxacin; LEV: Levofloxacin; CoN: Coagulase negative

Table 8: Prevalence of Extended Spectrum β lactamase Producing Bacterial Isolates

Bacterial Isolates	No of Isolates	ES β L Producers	Non ES β L Producers	χ^2	p-value
		No (%)	No (%)		
<i>E. coli</i>	62	27(43.5)	35(56.5)	3.78	0.437
<i>K. pneumoniae</i>	17	10(58.8)	7(41.2)		
<i>P. aeruginosa</i>	32	20(63.0)	12(38.0)		
<i>S. marcescens</i>	10	6(60.0)	4(40.0)		
<i>Enterobacter sp</i>	10	5(50.0)	5(50.0)		
Total	131	68(51.9)	63(48.1)		

Keys: ES β L: Extended Spectrum β lactamase; values in parenthesis represent percentages

DISCUSSION

Mid-stream urine (MSU) samples are among the numerous samples frequently sent to laboratories for microbiological analysis. In our study, the microscopic examinations of MSU samples showed the presence of leucocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin and glucose. Of the 150 MSU samples screened, 29.3 % had nitrites and the presence of nitrite in MSU samples in this study corroborated the findings of Akinjogunla *et al.* (2019). The detection of nitrite has a predictive value for UTIs since nitrite is formed as a metabolic product of bacteria that breakdown nitrate to nitrite (Akinjogunla *et al.*, 2019). The proportion of outpatients with proteinuria in this study was higher than the values obtained in United States of America (Akor *et al.*, 2009). Proteinuria in the samples could be a non-specific biomarker for UTIs (Akor *et al.*, 2009). Nitrites were always detected in urine samples for which Gram-negative bacteria were isolated. Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and *P. mirabilis* can reduce nitrate present in urine to nitrite, hence could account for the nitrate present. The occurrence of pus cell and yeast cells in the MSU samples in this study agreed with Onuoha and Fatokun (2014) who obtained pus cells and yeast cells in the MSU samples of patients in Afikpo, Ebonyi State, Nigeria.

In this study, female had the higher occurrence of SBU than the male and this finding was in agreement with studies carried out by Ibadin *et al.* (2006). The higher occurrence of SBU in females might be attributed to a variety of factors such as (i) physiological and anatomical differences in males and females (Swetha *et al.*, 2014), (ii) in-coordinate voiding of urine in female is often associated with constipation and encourages infection of the SBU (Swetha *et al.*, 2014), (iii) vaginal microflora also play a critical role in encouraging vaginal colonization with microorganisms leading to UTIs (Phipps *et al.*, 2006). In this study, the highest prevalence of SBU (42.9%) was found among subjects aged 21 to 30 yrs and this was in contrast to the findings of Turpin *et al.* (2007) whose highest prevalence of SBU (13%) was reported in the age group 35 to 39 yrs.

Numerous studies have revealed the geographical variability of pathogens occurrence in cases of significant bacteraemia among populations with the predominance of Gram - negative bacteria especially *E. coli* (Shill *et al.*, 2010; Tigist *et al.*, 2016; Akinjogunla *et al.*, 2019), while in some regions of the world, *S. aureus* had the highest prevalence of bacterial pathogen (Tosin *et al.*, 2018). In this study, *E. coli*, *K. pneumoniae*, *S. aureus*, *Streptococcus* spp, *P. aeruginosa* and *E. faecalis* were isolated from the MSU samples. The isolation of *E. coli*, *P. mirabilis* and *S. aureus* from the MSU samples was in agreement with findings of Ahmed *et al.* (2000) who reported the prevalence of *E. coli*, *Proteus* sp and *S. aureus* in MSU samples of patients with UTIs in Sudan. The prevalence of uropathogens such as *E. coli*, *Proteus* spp and *S. aureus* found in this study was in agreement with studies conducted in India and Sudan (Gonzalez and Schaeffer, 1999). [Studies in Nigeria also showed E. coli as the most common uropathogen \(Oluremi et al., 2011\). E. coli had the highest occurrence, followed by S. aureus in this study but differed from the reports of Okesola and Oni \(2009\) in South West, Nigeria.](#)

The results of the antibiotic susceptibility of the bacterial isolates from MSU samples of the patient showed diverse percentages of sensitivity and resistance. The high sensitivity of *S. aureus* and *E. coli* to ciprofloxacin in this study was similar to

the results of Ehinmidu (2003) but contradicted the results of Shill *et al.*, (2010) where *E. coli* was reported to be resistant to ciprofloxacin in the study conducted at Diagnostic Centers in Dhaka, Bangladesh. In our study, there was high sensitivity of the bacterial isolates to gentamycin and ofloxacin and this confirmed the reports of Mbata (2007) in Nsukka, Nigeria. Gentamycin is administered parenterally and, therefore, due to the discomfort of injection, it is less likely to be misused than oral drugs (Ngwai *et al.*, 2012). The occurrence of streptomycin resistant *S. aureus* and *E. coli* to in this study was in conformity with the results of Ehinmidu (2003). The findings on the antibiotic susceptibility of the bacteria from MSU in this study were also similar to the result of Shalini *et al.*, (2011) in India. The observed resistance to the antibiotics is a probable indication of earlier exposure of the isolates to these drugs and / or indiscriminate use of antibiotics among the undergraduate students, which has favoured the emergence of resistance strains. ES β L-producing organisms are known to exhibit important therapeutic implications as they show resistance against third-generation cephalosporins (Akinjogunla *et al.*, 2011). The occurrence of ES β L producing *E. coli* and *K. pneumoniae* isolated from MSU agreed with Osthoff *et al.*, (2015) who reported *E. coli* and *K. pneumoniae* producing ES β L in Australia. Asymptomatic urinary tract colonization might predispose to subsequent invasive infection with ES β L-Gram negative bacteria and many studies have described a significantly increased risk for invasive infection with ES β L-Gram negative bacteria in patients (Reddy *et al.*, 2007; Su *et al.*, 2010; Osthoff *et al.*, 2015). In this study, *S. marcescens* produced ES β L and this corroborated the results of Su *et al.*, (2010) who obtained ES β L producing *S. marcescens* using antibiogram based method.

Conclusion

This study has shown the antibiogram and occurrence of ES β Ls of bacterial isolates in MSU and also revealed the necessity to routinely carry out medical examinations of subjects attending various hospitals for asymptomatic bacteriuria so as to reduce the rate of SBU and prevent symptomatic infection and its complications.

REFERENCES

- Akinjogunla, O. J., Eghafona, N. O. and Enabulele, I. O. (2011). Aetiological agents of acute otitis media (AOM): prevalence, antibiotic susceptibility, β -Lactamase (β) and extended spectrum β -lactamase (ES β Ls) production. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(3):333-353.
- Akinjogunla, O. J., Odeyemi, A. T., and Olasehinde, G. I. (2010). Epidemiological Studies of Urinary Tract Infection (UTI) among Post-menopausal Women in Uyo Metropolis, South South, Nigeria. *Journal of American Science*, 6(12):1674-1681.
- Akinjogunla, O. J., and Divine-Anthony, O. (2013). Asymptomatic Bacteriuria among Apparently Healthy Undergraduate Students in Uyo, South South, Nigeria. *Annual Review and Research in Biology*, 3(3):213-225.
- Akinjogunla, O. J., Umo, N. A and Udoh, M. D. (2019). Slime producing multidrug resistant bacterial isolates and dipstick assay for nitrite and leucocyte esterase in urine specimens of antenatal patients in Uyo, Nigeria. *Journal of Experimental Research*, 7(3):44-52.

- Akor, F., Okolo, S. N., Agaba, E. I., and Okolo, A. (2009). Urine examination findings in apparently healthy new school entrants in Jos, Nigeria. *South African Journal of Child Health*, 3:60-63.
- Ahmed, A. A., Osman, H., Mansour, A. M., Musa, H. A., Ahmed, A. B., Karrar, Z., and Hassan, H. S. (2000). Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infection in Sudan. *American Journal of Tropical Medicines and Hygiene*, 63:259–263.
- Brauner, A., Flodin, U., Hylander, B. and Ostenson, C. G. (1993). Bacteriuria, bacterial virulence and host factors in diabetic patients. *Diabetic medicine: A Journal of the British Diabetic Association*, 10(6):550–554.
- Cheesbrough M. (2006). *District Laboratory Practice in Tropical Countries*. Cambridge University Press.
- Clinical and Laboratory Standards Institute (2015). Performance Standards for Antimicrobial Disk Susceptibility Tests. 472 M012-A12. Clinical and Laboratory Standards Institute, Wayne, PA.
- Ehinmidu, J. O. (2003). Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria,” *Tropical Journal of Pharmaceutical Research*, 2(2):223–228.
- Federal Republic of Nigeria Official Gazette (FRNOG) (2007). Legal Notice on Publication of the Details of the Breakdown of the National and State Provisional Totals 2006 Census.
- Gonzalez, C. M. and Schaeffer, A. J. (1999). Treatment of urinary tract infection: what's old, what's new, and what works. *World Journal of Urology*, 6:372-382.
- Ibadin, O. M, Onunu, A. and Ukoh, G. M. (2006). Urinary tract infection in adolescent/young adult Nigeria with acquired human immuno-deficiency disease in Benin City. *Journal of Medicine and Biomedical Research*, 5:55-60.
- Mbata, M. (2007). Prevalence and Antibiogram of Urinary Tract Infections among Prison Inmates in Nigeria. *Internet Journal of Microbiology*, 3:10-15.
- Ngwai, Y. B., I. Halima, Y. Elijah, and Owuna, G. (2012). Bacteriuria and Antimicrobial Susceptibility of *Escherichia coli* Isolated from Urine of Asymptomatic University Students in Keffi, Nigeria”. *Jundishapur Journal of Microbiology*, 5(1):323-327.
- Okesola, A. O. and Oni, A. A. (2009). Antimicrobial resistance among common bacteria pathogens in South West, Nigeria. *American Journal Agricultural Environmental Science*, 5(3):327-330.
- Oluremi, B. B., Idowu, A. O., and Olaniyi, J. F. (20011). Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a teaching hospital in Southwestern Nigeria. *African Journal of Microbiology Research*, 5(22):3658-3663.
- Osthoff, M., McGuinness, S. L., Wagen, A. Z., and Eisen, D. P. (20015). Urinary tract infections due to extended-spectrum beta-lactamase-producing Gram-negative bacteria: identification of risk factors and outcome predictors in an Australian tertiary referral hospital. *International Journal of Infectious Diseases*, 34:79–83.
- Phipps, M. and Petricevic, S. (2006). The tendency of individuals to transfer DNA to handled items. *Forensic Science International*, 168(2-3):162-168.
- Redder, J. D., Leth, R. A. and Moller, J. K. (2016). Analyzing risk factors for urinary tract infection based on automated monitoring of hospital-acquired infection. *Journal of Hospital Infection*, 92:3397- 4000.
- Reddy, P., Malczynski, M., Obias, A., Reiner, S., Jin, N., Huang, J., Noskin G. A, and Zembower, T. (2007). Screening for Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae among High-Risk Patients and Rates of Subsequent Bacteremia. *Clinical Infectious Diseases*, 45(7):846-852.
- Shalini, D., Joshi, M. C., Rashid, M. K., and Joshi, H. S. (2011). Study of Antibiotic Sensitivity Pattern in Urinary Tract Infection at a Tertiary Hospital. *National Journal of Integrated Research in Medicine*, 2(3):43-46.
- Shill, M. C., Huda, N. H., Moain, F. B., and Utpal, K., Karmakar, U. K. (2010). Prevalence of Uropathogens in Diabetic Patients and their Corresponding Resistance Pattern: Results of a Survey Conducted at Diagnostic Centers in Dhaka, Bangladesh. *Oman Medical Journal*, 25(4): 282–285.
- Smith, M. B. H. (1994). Screening for urinary tract infection in asymptomatic infants and children. *Canadian Guide to Clinical Preventive Health Care*: 220-230.
- Stamm, W. E, and Hooton, T. M. (1993). Management of UTIs in adults. *The New England Journal of Medicine*, 239:1328-1334.
- Onuoha, S. C., and Fatokun, K. (2014). Prevalence and Antimicrobial Susceptibility Pattern of Urinary Tract Infection (UTI) among Pregnant Women in Afikpo, Ebonyi State, Nigeria. *American Journal of Life Sciences*, 2(2):46-52.
- Su, P. A., Wu, L. T., Cheng, K. C., Ko, W. C., Chuang, Y. C., and Yu, W. L. (2010). Screening extended-spectrum beta-lactamase production in *Enterobacter cloacae* and *Serratia marcescens* using antibiogram-based methods. *Journal of Microbiology Immunology and Infection*, 43(1):26-34.
- Swetha, V. V., Rao, U. S., Prakash, P. H., and Subbarayudu, S. (2014). Aerobic bacteriological profile of urinary tract infections in a tertiary care hospital. *International Journal of Current Microbiology and Applied Science*, 3(3):120-125.
- Syed, S. A., Ali, S., Abdulaziz, A. A., Ibrahim, H. B. and Bard, N. A. (2019). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infection. *International Journal of Health Sciences. (Quassim)*, 13(2):48-55.
- Tigist, G., Belayneh, R., Aseer, M., Mohammedaman, M., and Behailu, M. (2016). Prevalence, diversity and antimicrobial resistance of bacteria isolated from the UTI patients of Arba Minch Province, Southern Ethiopia. *Translational Biomedicine*, 7:3.
- Tosin T. O., Akinwumi, A. A., Adeyemi, O. and Oluseyi, A. A. (2018). Urinary tract infections and antibiotic sensitivity pattern of uropathogens in a tertiary hospital in south west, Nigeria. *Sahel Medical Journal*, 21:18-22.
- Turpin, C. A., Minkah, B., Danso, K. A., and Frimpong, E. H. (2007). Asymptomatic bacteriuria in pregnant women attending antenatal clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. *Ghana Medical Journal*, 41(1):26–29.