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Full Length Research Article

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

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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 (ESβL) 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. ESβLs 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, prostitis 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 occurence 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 (ESβLs) producing bacterial isolates have spread worldwide and have become endemic in several countries (Akinjogunla *et al*., 2011; Osthoff *et al.*, 2015). The ESβLs are frequently plasmid mediated and derived from mutations in the classic Temoria (TEM) and Sulphydyl 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 ESβLs production of bacteria isolated.

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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 5º 02' 37" North and longitudes 7º 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 ≥ 60yrs, 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 37ºC. Then the culture colonies were observed and counted using Digital colony counter (Model: LT-37). Colony counts yielding bacterial growth of ≥ 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 37ºC. 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, 10µg) ; Ceftazidime (CAZ, 30 µg); Streptomycin (STP, 30µg) ; Ciprofloxacin (CPF, 5µg) ; Gentamycin (GEN, 10µg) ; Ofloxacin (OFL, 5µg) ; Ceftriaxone (CEF, 30µg) and Cotrimoxazole (COT, 30µg), while the Gram negative disc were : Ofloxacin (OFL, 5µg) ; Reflacine (PEX, 5 µg) ; Augmentin (AU, 30µg) ; Ciprofloxacin (CPX, 5µg); Cotrimoxazole (SXT, 25µg); Gentamycin (GEN, 10µg) ; Cephalothin (CEP, 30µg) ; Streptomycin (S, 10µg) ; Nalidixic Acid (NA, 5µg) and Ampicillin (PN, 10µg) (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 βetalactamase (ESβL) Producing Bacterial Isolates

The ESβL 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 µg) was placed at the center of the MHA plate and cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) were placed on either side of the central disc (AMC, 20/10 µg) 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 ESβL 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 ≤0.05 were determined using chi-square ($\chi 2$) 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 ≥ 61yrs 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

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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 ≥ 61yrs 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 ≥ 61yrs. 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 ≥ 61yrs 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 ESβL producers, while 48.1% were non EsβLs producers. ESβLs 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 ESβ|L producing bacterial isolates from MSU samples was not statistically significant from the non-ESβ|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	χ2	p-values
Age (yrs)					
≤ 20	21	4	19.0		
21-30	28	12	42.9		
31-40	22	8	36.4		
41-50	27	11	40.7	3.88	0.567
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		
Married	61	16	26.2	7.31	0.063
Divorced	5	1	20.0		
Widowed	17	4	23.5		
Educational Level					
Primary School	18	5	27.8		
Secondary School	38	13	34.2	0.474	0.789
Tertiary	94	34	36.2		
Occupation					
Farmers	4	1	25.0		
Public Servant	14	3	21.4		
Traders	22	5	22.7	1.42	0.134
Civil Servants	34	9	26.5		
Students	76	34	44.7		

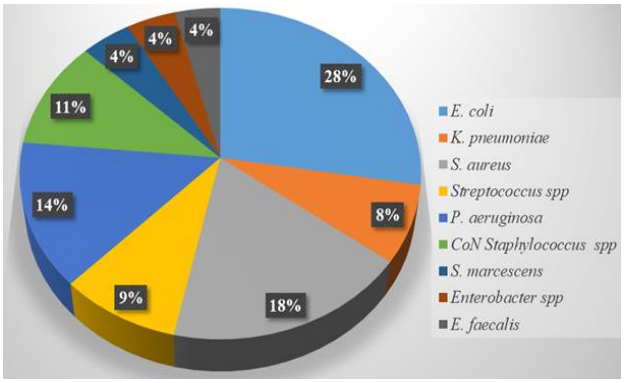

Fig 1: Percentage Occurrence of Bacterial Isolates from Mid-stream Urine Samples of Out-patients

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

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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 (%)
E. coli	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)
K. pneumoniae	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)
P. aeruginosa	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)
S. marcescens	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)
Enterobacter spp	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)
E. faecalis	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 (%)
S. aureus	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)
Streptococcus sp	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-*Staphylococcus* sp	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

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

Bacterial Isolates	Resistance Pattern	No (%) of Occurrences
E. coli	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)
K. pneumoniae	OFX-S-SXT	2(11.8)
	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)
S. marcescens	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)
Enterobacter 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)
P. aeruginosa	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

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

Bacterial Isolates	Resistance Pattern	No (%) of Occurrences
E. faecalis	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)
S. aureus	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)
Streptococcus sp	CPX-NB-S-RD-CH-APX-LEV	4(20.0)
	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-*Staphylococcus* sp	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 βetalactamase Producing Bacterial Isolates

Bacterial Isolates	No of Isolates	ESβL Producers No (%)	Non ESβL Producers No (%)	χ2	p-value
E. coli	62	27(43.5)	35(56.5)		
K. pneumoniae	17	10(58.8)	7(41.2)		
P. aeruginosa	32	20(63.0)	12(38.0)	3.78	0.437
S. marcescens	10	6(60.0)	4(40.0)		
Enterobacter sp	10	5(50.0)	5(50.0)		
Total	131	68(51.9)	63(48.1)		

Keys: ESβL: Extended Spectrum βetalactamase; values in parenthesis represent percentages

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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 bacteurria 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 occurence, 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. ESβL-producing organisms are known to exhibit important therapeutic implications as they show resistance against third-generation cephalosporins (Akinjogunla *et al*., 2011). The occurrence of ESβL producing *E. coli* and *K. pneumoniae* isolated from MSU agreed with Osthoff *et al*., (2015) who reported *E. coli* and *K. pneumoniae* producing ESβL in Australia. Asymptomatic urinary tract colonization might predispose to subsequent invasive infection with ESβL-Gram negative bacteria and many studies have described a significantly increased risk for invasive infection with ESβL-Gram negative bacteria in patients (Reddy *et al*., 2007; Su *et al.,* 2010; Osthoff *et al*., 2015). In this study, *S. marcescens* produced ESβL and this corroborated the results of Su *et al.,* (2010) who obtained ESβL producing *S. marcescens* using antibiogram based method.

Conclusion

This study has shown the antibiogram and occurrence of ESβLs 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.

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