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Slime Producing Multidrug Resistant Bacterial Isolates and Dipstick Assay for Nitrite And Leucocyte Esterase in Urine Specimens of Antenatal Patients in Uyo, Nigeria.

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ABSTRACT

The slime producing multidrug resistant bacteria isolates and dipstick assay for nitrite and leucocyte esterase in the mid-stream urine (MSU) specimens of antenatal patients were determined using standard bacteriological technique, Congo red agar and urine dipsticks. Of the 245 MSU specimens collected from the subjects, 30.2 % had bacterial counts ≥ 10^5 CFUml^{-1}, while 69.8 % MSU had bacterial counts 10^5 CFUml^{-1}. The highest significant bacteriuria (SBU) was obtained among the subjects aged 21-30 yrs, while the subjects aged ≥41 yrs had the lowest SBU. A total of 30 (12.2 %) and 42 (17.1 %) MSU were positive for nitrite and leucocyte esterase, respectively. The MSU specimens with the highest SBU and occurrence of nitrite and leucocyte esterase were obtained from the subjects in their second trimester. The subjects in their first trimester harboured the highest numbers of bacterial isolates (n=135), while those in their third trimester had the lowest numbers (n=112). The slime producing bacterial isolates in decreasing order was CoN- Staphylococcus spp (18.0%) ≥ S. aureus (15.0%) S. pyogenes / E. coli ≥ (14.0%) K pneumoniae (12.0%) ≥ P. aeruginosa (11.0%) E. faecalis (9.0%) ≥ Proteus spp (7.0%). S. aureus were highly ≥ sensitive to Ceftriaxone; S. pyogenes were moderately sensitive to Nalidixic acid; ≥75.5 % E. coli showed sensitivity to Ciprofloxacin, ≥ 72.0 % Proteus spp were sensitive to Gentamycin, while between 33.3 % and 41.7 % S. pyogenes and E. faecalis were resistant to Amoxicillin and Erythromycin. Of the 376 isolates, 107 were non-MDR isolates, while 215 were MDR isolates. Among the MDR isolates, 113 isolates were resistant to ≤ 4 antibiotics, while 102 isolates were resistant to between 5 and 10 antibiotics. The findings of this study showed the needs to continuously monitor the antibiotic susceptibility profiles of slime producing bacteria implicated in UTI and also endeavor to avert and/or curtail slime-associated infections.

Key Words: Antenatal, Trimester, Slime, Antibiotics, Nitrite, Leucocyte Esterase, Uyo.

INTRODUCTION

Slime is a viscous, loosely bound, extracellular material consisting of exopolysaccharides, glycoproteins and glycolipids (Akinjogunla *et al*. 2018). Slime production has been considered as a significant virulence factor of some bacterial strains (Ammendolia *et al.* 1999). Slime production facilitates bacterial adherence to smooth surfaces such as prosthetic medical devices and catheters; plays a role in the establishment and severity of infection (Ammendolia *et al.* 1999), and protects bacteria from desiccation and antibiotics, thus, resulting in antimicrobial resistance (Akyar *et al.* 1998; Ammendolia *et al.* 1999). Bacterial slime has been associated with sepsis, including intravenous-catheter-related bacteremia and other prosthetic device infections (Rupp and Archer, 1994).

The urinary tract infection (UTI) and its associated complications are the cause of nearly 150 million deaths per year worldwide (Amiri *et al.* 2015). The apparent reduction in immunity of pregnant women encourages the growth of microorganisms and the physiological changes that occur in urinary tracts during pregnancy can cause healthy women to be more susceptible to serious complications due to UTI. The urinary tract infections are the most common bacterial infections during pregnancy accounting for approximately 10 % of hospital visits (Millar and Cox, 1997). These infections can be asymptomatic or symptomatic bacteriuria occurring in 5–10% and 1–3 % among pregnant women, respectively (Gilstrap and Ramin, 2001). Some patients with untreated asymptomatic bacteriuria develop symptomatic cystitis (Barnick and Cardozo, 1991). Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low birth weight, increased morbidity and mortality for mother and child (Blomberg et al. 2005; Macejko and Schaeffer, 2007). *Escherichia coli* and species of *Klebsiella*, *Staphylococcus* and *Enterococcus* have been reported as the cause of UTI among pregnant women (Delzell and Lefevre, 2000; Gilstrap and Ramin, 2001).

Many parameters such as bilirubin, glucose, blood, nitrites and leucocyte esterase may be detected in the urine samples. The presence of each of these parameters in urine has its clinical significance and several studies have linked the presence of nitrite and leucocyte esterase to significant bacteriuria (Charles, 2011; Ratna and Sharan, 2017). Nitrites are only found in urine when uropathogenic bacteria, especially Gram-negative rod bacteria in the family enterobacteriaceae, convert urinary nitrates to nitrites, while the leucocyte esterase assay is an indirect measure of pyuria as it detects the production of this enzyme by the host's polymorphonuclear cells (Semeniuk and Church, 1999). In this study the occurrence of slime producing multidrug resistant bacteria isolates and dipstick assay for nitrite and leucocyte esterase in the MSU specimens of antenatal patients were determined.

MATERIALS AND METHODS

Collection of Specimens

A total of 245 clean-catch mid-stream urine (MSU) specimens were aseptically collected using sterile containers from pregnant women (aged ≤ 20 to ≥ 40 yrs), at different gestational age/trimesters, attending antenatal clinics between October, 2017 and September, 2018 in Uyo, Nigeria. Verbal informed consent was obtained from each pregnant woman who had not received antibiotic treatment for the previous one week prior to specimen collection. The MSU specimens were transported in cooler boxes to Microbiology Laboratory, University of Uyo, for bacteriological analysis within 1-4 hrs of collection.

Dipstick Test for Nitrite and Leukocyte Esterase

The nitrite and leukocyte esterase in MSU specimens of pregnant women were detected using urine dipsticks (Medi-Test Combi 10 SGL, Macherey-Nagel, Germany). The dipstick was dipped into fresh uncentrifuged MSU specimen and left for approximately 5 secs before removing it. A colour change of the dipstick from colourless to pink or red within 60 secs indicated nitrites, while a colour change of the dipstick from off-white to purple within 2 mins indicated the presence of leucocyte esterase.

Bacteriology of the Urine Specimens

Each of the uniformly mixed, uncentrifuged, MSU specimens was aseptically inoculated onto dried plate of Cysteine Lactose Electrolyte Deficient (CLED) agar using a sterile calibrated drop that delivered 0.002 ml of urine specimen. The plates were aerobically incubated at 37 °C for 24 hrs. After incubation, the colonies on each plate were observed, enumerated and counts of >10^5 CFU/ml were considered as significant bacteriuria (SBU). The cultures with significant growth were further subcultured onto plates of nutrient agar, aerobically incubated at 37°C for 24 hrs, maintained on nutrient agar slant at 4°C, characterized and identified using their colonial appearances, Gram staining reaction, biochemical and sugar fermentation tests.

Antibiotic Susceptibility Testing of Bacterial Isolates

In vitro susceptibility of the bacterial isolates to Ampicillin (AMP, 10 µg), Tetracycline (TET, 30 µg),_Ceftriaxone (CTX, 30 µg), Nalidixic acid (NA, 30 µg), Ciprofloxacin (CIP, 5 µg), _Gentamycin (GEN, 10 µg), Nitrofurantoin (NIT, 30o µg), Amoxicillin (AMO, 25 µg), Erythromycin (ERY, 15 µg) and Chloramphenicol (CHL, 3 µg) (Oxoid, UK) was determined by disc diffusion method (CLSI, 2005). Ten microliters (10 µL) of each bacterial isolate, prepared directly from an overnight agar plate and adjusted to 0.5 McFarland Turbidity Standard, was inoculated onto each plate of Mueller Hilton Agar (MHA). The antibiotic discs were aseptically placed on the surfaces of the culture plates with a sterile forceps and gently pressed to ensure even contact. The plates were

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incubated at 37 °C for 18 hr; inhibitory zones were observed and measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the standard interpretative zone sizes given by CLSI guidelines (CLSI, 2005).

Determination of Multiple Antibiotic Resistance Index

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=*x/y*, where *'x'* was the number of antibiotics to which test isolate displayed resistance and *'y'* was the total number of antibiotics to which the test isolates has been evaluated for sensitivity. Isolates that were resistant to three or more antibiotics were taken to be multiple antibiotic resistant (Jan *et al.* 2002; Akinjogunla *et al.* 2017).

Detection of Slime Producing Bacterial Isolates

Slime producing *bacterial* isolates were detected using Congo red gar. The Congo red stain, prepared as a concentrated aqueous solution, was autoclaved at 121 °C for 15 mins. The brain heart infusion (BHI) agar with sucrose was also prepared and autoclaved at 121 °C for 15 mins. The Congo red stain was added to the autoclaved BHI agar with sucrose at 55 °C. Each of the test isolates was streaked on each Congo red agar plate and aerobically incubated for 24-48 hrs at 37 ºC. Formation of black colonies with dry crystalline consistency indicated slime production (Freeman *et al.* 1989; Akinjogunla *et al.* 2018).

RESULTS

The occurrence of nitrite, leucocyte esterase and significant bacteriuria (SBU) in MSU specimens of pregnant women (n=245) based on ages and trimesters are presented in Tables 1 and 2. Of the 245 MSU specimens collected from the pregnant women, 74 (30.2 %) had bacterial counts ≥ 10^5 $CFUml^{-1}$; indicating significant bacteriuria (SBU), while 171 (69.8 %) MSU specimens had bacterial counts ≤ 10^5 $CFUml^{-1}$. The highest SBU was obtained among the pregnant women aged 21-30 yrs (n=25, 38.5 %), while the pregnant women aged ≥ 41 yrs had the lowest SBU (n=11, 19.3 %). A total of 30 (12.2 %) and 42 (17.1 %) MSU specimens were positive for only nitrite and leucocyte esterase respectively, while 20 (8.2 %) MSU specimens were positive for both nitrite and leucocyte esterase (Table 1). Regarding the trimester, the highest proportion of MSU specimens positive for nitrite (16/81, 19.8 %), leucocyte esterase (22/81, 27.2 %) and also with the highest SBU (31/81, 38.3 %) were obtained from the pregnant women in the second trimester, followed by those in their third trimester (23/75, 30.7 %) and first trimester (20/89, 22.5 %), respectively (Table 2)

The occurrences of bacterial isolates in the MSU cultures of the pregnant women with respect to trimesters and ages are shown in Table 3. The pregnant women in the first trimester harboured the highest proportion of bacterial isolates (n=135, 35.9 %), followed by the pregnant women in the second trimester with 34.3 % (n=129) isolates, while those in the third trimester had the lowest proportion (n=112, 29.8 %). The MSU cultures of the pregnant women had seven bacterial genera comprising *Escherichia*, *Staphylococcus*, *Streptococcus*, *Proteus*, *Pseudomonas*, *Klebsiella* and *Enterococcus* (Table 3). With respect to the ages of the subjects, the predominant bacterial isolate was *E. faecalis*, *S. pyogenes*, CoN *Staphylococcus* spp and *K. pneumoniae* for age group ≤ 20 yrs, 21-30 yrs, 31-40 yrs and 41 yrs, respectively
(Table 3).

The percentage occurrence of slime producing urinary bacterial isolates in decreasing order was as follows: CoN- *Staphylococcus* spp (18.0%) ≥ *S. aureus* (15.0%) *S. pyogenes / E. coli* (14.0%) ≥ *K pneumoniae* (12.0%) *P. aeruginosa* (11.0%) ≥ *E. faecalis* (9.0%) *Proteus* spp (7.0%) (Fig 1).

The results of the marked variabilities in the antibiotic sensitivity profiles of the urinary bacterial isolates are shown in Tables 4 and 5. Ciprofloxacin and Ceftriaxone showed high antibiotic activities against *S. aureus*, CoN-*Staphylococcus* spp and *E. faecalis* with percentage sensitivities ranging from 66.7 % to 80.0%; *S. pyogenes* were moderately sensitive to Nalidixic acid, Gentamycin and Ceftriaxone with percentage sensitivities ranging from 54.2 % to

58.3 %, while between 33.3 % and 41.7 % *S. pyogenes* and *E. faecalis* were resistant to Amoxicillin and Erythromycin (Table 4). *Escherichia coli*, the predominant isolate among the Gram negative urinary bacteria obtained, were highly sensitive to Ceftriaxone (75.5 %), Amoxicillin (71.7 %) and Ciprofloxacin (77.4 %). More than 72.0 % *Proteus* spp and *K pneumoniae* were sensitive to Gentamycin, while ≥ 31.7 % *E. coli*, *Proteus* spp., *P. aeruginosa* and *K pneumoniae* were resistant to Tetracycline (Table 5).

The multiple antibiotic resistance (MAR) indices of resistant bacterial isolates are shown in Table 6. Fifty-four (14.4%) isolates were sensitive to all the antibiotics, 107 (13.3%) were non-MDR isolates, while 215 (57.2%) were MDR isolates. Of the 215 MDR isolates, 67 isolates were resistant to three antibiotics, 46 isolates were resistant to four antibiotics, while 102 isolates were resistant to between 5 and 10 antibiotics. The MDR *E. faecalis*, *Proteus* spp and *P. aeruginosa* had MAR indice ranging from of 0.3 to 0.8, while the MAR indice of *K. pneumoniae and* CoN *Staphylococcus* spp ranged from 0.3 to 0.5 (≤ 5 antibiotics) and 0.3 to 0.7 (≤ 7 antibiotics), respectively (Table 6).

Table 1: Occurrence of Nitrite, Leucocyte Esterase and Significant Bacteriuria in Urine Specimens of Pregnant women Based on Ages

Age (Yrs)	No of Specimens Collected	Specimens without SBU No (%)	Specimens with SBU No (%)	Specimens with NIT No (%)	Specimens with LE No (%)	Specimens with NIT + LE No (%)
≤ 20	41	26 (63.4)	15 (36.6)	6 (14.6)	8 (19.5)	2 (4.9)
21-30	65	40 (61.5)	25 (38.5)	12 (18.5)	18 (27.7)	10 (15.4)
31-40	82	59 (72.0)	23 (28.0)	8 (9.8)	12 (14.6)	6 (7.3)
≥ 41	57	46 (80.7)	11 (19.3)	4 (7.0)	4 (7.0)	2 (3.5)
Total	245	171 (69.8)	74 (30.2)	30 (12.2)	42 (17.1)	20 (8.2)

Keys: SBU: Significant Bacteriuria; NIT: Nitrite; LE: Leucocyte Esterase; Values in parenthesis represent percentages

Table 2: Occurrence of Nitrite, Leucocyte Esterase and Significant Bacteriuria in Urine Specimens of Pregnant women Based on Trimesters

Trimester	No of Specimens Collected	Specimens without SBU No (%)	Specimens with SBU No (%)	Specimens with NIT No (%)	Specimens with LE No (%)	Specimens with NIT + LE No (%)
1	89	69 (77.5)	20 (22.5)	6 (6.7)	8 (9.0)	3 (3.4)
2	81	50 (61.7)	31 (38.3)	16 (19.8)	22 (27.2)	11 (13.6)
3	75	52 (69.3)	23 (30.7)	8 (10.7)	12 (16.0)	6 (8.0)
Total	245	171 (69.8)	74 (30.2)	30 (12.2)	42 (17.1)	20 (8.2)

Keys: SBU: Significant Bacteriuria; NIT: Nitrite; LE: Leucocyte Esterase; Values in parenthesis represent percentages

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Table 3: Occurrence of Bacterial Isolates in Urine Specimens of Pregnant Women Based on Trimesters and Ages

Bacterial Isolates	Trimester 1st No (%)	Trimester 2nd No (%)	Trimester 3rd No (%)	Age (Yrs) ≤ 20 No (%)	Age (Yrs) 21-30 No (%)	Age (Yrs) 31-40 No (%)	Age (Yrs) ≥41 No (%)	Total No (%)
E. coli	37 (34.9)	36 (34.0)	33 (31.1)	17 (16.0)	28 (26.4)	33 (31.1)	28 (26.4)	106(28.2)
S. aureus	20 (27.0)	29 (39.2)	25 (33.8)	14 (18.9)	22 (29.7)	23 (31.1)	15 (20.3)	74(19.7)
Proteus spp	8 (24.2)	10 (30.3)	15 (45.5)	6 (18.2)	9 (27.3)	8 (24.2)	10 (30.3)	33(8.8)
S. pyogenes	21 (43.8)	15 (31.3)	12 (25.0)	10 (20.8)	15 (31.3)	14 (29.2)	9 (18.8)	48(12.8)
CoN *Staphylococcus* spp	15 (60.0)	10 (40.0)	0 (0.0)	5 (20.0)	5 (20.0)	12 (48.0)	3 (12.0)	25(6.6)
P. aeruginosa	14 (34.1)	17 (41.5)	10 (24.4)	8 (19.5)	11 (26.8)	16 (39.0)	6 (14.6)	41(10.9)
K. pneumoniae	5 (31.3)	4 (25.0)	7 (43.8)	2 (12.5)	3 (18.8)	2 (12.5)	9 (56.3)	16(4.3)
E. faecalis	15 (45.5)	8 (24.2)	10 (30.3)	8 (24.2)	8 (24.2)	11 (33.3)	6 (18.2)	33(8.8)
Total	135 (35.9)	129 (34.3)	112 (29.8)	70(18.6)	101(26.9)	119(31.6)	86(22.9)	376(100)

Keys**:** CoN: Coagulase negative; Values in parenthesis represent percentages

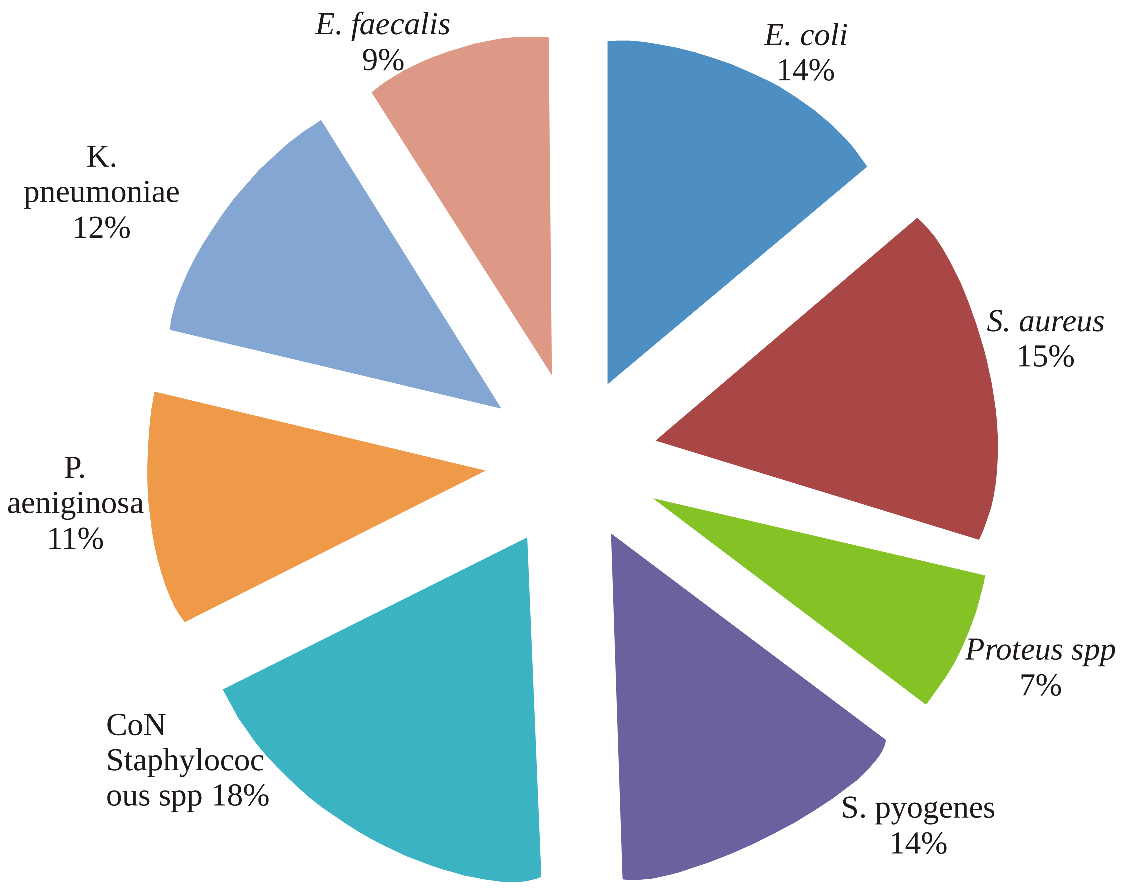

Fig I: Percentage Occurrences of Slime Producing MDR-Bacterial Isolates from Urine Specimens of Pregnant Women

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Table 4: Antibiotic Susceptibility Profile of Gram Positive Urinary Bacterial Isolates from Pregnant Women

Antibiotics	*S. aureus* (n=74)			*S. pyogenes* (n=48)			CoN- *Staphylococcus* spp (n=25)			*E. faecalis* (n=33)		
	S	I	R	S	I	R	S	I	R	S	I	R
	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %
Nitrofurantoin	46 (62.2)	8 (10.8)	20 (27.0)	34 (70.8)	6 (12.5)	8 (16.7)	18 (72.0)	6 (24.0)	1 (4.0)	18 (54.5)	6 (18.2)	9 (27.3)
Amoxicillin	38 (51.4)	24 (32.4)	12 (16.2)	18 (37.5)	10 (20.8)	20 (41.7)	10 (40.0)	6 (24.0)	9 (36.0)	20 (60.6)	2 (6.0)	11 (33.3)
Nalidixic Acid	52 (70.3)	8 (10.8)	14 (18.9)	28 (58.3)	8 (16.7)	12 (25.0)	10 (40.0)	0 (0.0)	15 (60.0)	12 (36.4)	12 (36.4)	9 (27.3)
Ampicillin	36 (48.6)	16 (21.6)	22 (29.7)	22 (45.8)	14 (29.2)	12 (25.0)	14 (56.0)	4 (16.0)	7 (28.0)	18 (54.5)	10 (30.3)	5 (15.2)
Ciprofloxacin	52 (70.3)	12 (16.2)	10 (13.5)	30 (62.5)	4 (8.3)	14 (29.2)	20 (80.0)	4 (16.0)	1 (4.0)	24 (72.7)	2 (6.0)	7 (21.2)
Erythromycin	42 (56.8)	8 (10.8)	24 (32.4)	18 (37.5)	14 (29.2)	16 (33.3)	4 (16.0)	12 (48.0)	9 (36.0)	20 (60.6)	2 (6.0)	11 (33.3)
Gentamycin	52 (70.3)	12(16.2)	10 (13.5)	26 (54.2)	6 (12.5)	16 (33.3)	16 (64.0)	2 (8.0)	7 (28.0)	26 (78.8)	6 (18.2)	1 (3.0)
Ceftriaxone	56 (75.7)	6 (8.1)	12 (16.2)	28 (58.3)	8 (16.7)	12 (25.0)	18 (72.0)	6 (24.0)	1 (4.0)	22 (66.7)	4 (12.1)	7 (21.2)
Chloramphenicol	50 (67.6)	14 (18.9)	10 (13.5)	14 (29.2)	16 (33.3)	18 (37.5)	14 (56.0)	0 (0.0)	11 (44.0)	24 (72.7)	0 (0.0)	9 (27.3)
Tetracycline	40 (54.1)	20 (27.0)	14 (18.9)	18 (37.5)	10 (20.8)	20 (41.7)	16 (64.0)	4 (16.0)	5 (20.0)	14 (42.4)	8 (24.2)	11 (33.3)

Keys: S: Sensitive; I: Intermediate; R: Resistant; CoN: Coagulase Negative; Values in parenthesis represent percentages.

Table 5: Antibiotic Susceptibility Profile of Gram Negative Urinary Bacterial Isolates from Pregnant Women

Antibiotics	*E. coli* (n = 106)			*Proteus* spp (n = 33)			*P. aeruginosa* (n=41)			*K. pneumoniae* (n =16)		
	S	I	R	S	I	R	S	I	R	S	I	R
	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %	No %
Ceftriazone	80 (75.5)	14 (13.2)	12 (11.3)	18 (54.5)	6 (18.2)	9 (27.2)	28 (68.3)	6 (14.6)	7 (17.1)	8 (50.0)	2 (12.5)	6 (37.5)
Gentamycin	74 (69.8)	10 (9.4)	22 (20.8)	24 (72.7)	8 (24.2)	1 (3.0)	22 (53.7)	8 (19.5)	11 (26.8)	12 (75.0)	0 (0.0)	4 (25.0)
Nitrofurantoin	58 (54.7)	20 (18.9)	28 (26.4)	16 (48.5)	4 (12.1)	13 (39.4)	16 (39.0)	8 (19.5)	17 (41.5)	10 (62.5)	6 (37.5)	0 (0.0)
Nalidixic Acid	58 (54.7)	16 (15.1)	32 (30.2)	20 (60.6)	2 (6.1)	11 (33.3)	24 (58.5)	2 (4.9)	15 (36.6)	10 (62.5)	2 (12.5)	4 (25.0)
Chloramphenicol	48 (45.3)	20 (18.9)	38 (35.8)	22 (66.7)	0 (0.0)	11 (33.3)	12 (29.3)	12 (29.3)	17 (41.5)	8 (50.0)	0 (0.0)	8 (50.0)
Amoxicillin	76 (71.7)	6 (5.7)	24 (22.6)	12 (36.4)	4 (12.1)	17 (51.5)	12 (29.3)	14 (34.1)	15 (36.6)	6 (37.5)	4 (25.0)	6 (37.5)
Erythromycin	54 (50.9)	26 (24.5)	26 (24.5)	16 (48.5)	6 (18.2)	11 (33.3)	22 (53.7)	8 (19.5)	11 (26.8)	8 (50.0)	4 (25.0)	4 (25.0)
Tetracycline	42 (39.6)	22 (20.8)	42 (39.6)	12 (36.4)	8 (24.2)	13 (39.4)	18 (43.9)	10 (24.4)	13 (31.7)	4 (25.0)	6 (37.5)	6 (37.5)
Ciprofloxacin	82 (77.4)	10 (9.4)	14 (13.2)	20 (60.6)	4 (12.1)	9 (27.2)	28 (68.3)	4 (9.8)	9 (22.0)	14 (87.5)	0 (0.0)	2 (12.5)
Ampicillin	60 (56.6)	24 (22.6)	22 (20.8)	20 (60.6)	6 (18.2)	7 (21.2)	20 (48.8)	10 (24.4)	11 (26.8)	4 (25.0)	6 (37.5)	6 (37.5)

Keys: S = Sensitive, I = Intermediate, R = Resistant; Values in parenthesis represent percentages.

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Table 6: Multidrug Resistant Index of Bacterial Isolates from Urine Specimens of Pregnant Women

MAR Index	SA No (%)	SP No (%)	CS No (%)	EF No (%)	EC No (%)	PS No (%)	PA No (%)	KP No (%)	Total No (%)
0.0	9 (12.2)	5 (10.4)	4 (16.0)	6 (18.2)	15 (14.2)	8 (24.2)	4 (9.8)	3 (18.8)	54 (14.4)
0.1	9 (12.2)	7 (14.6)	3 (12.0)	3 (9.1)	13 (12.3)	5 (15.2)	6 (14.6)	4 (25.0)	50 (13.3)
0.2	11 (14.9)	6 (12.5)	3 (12.0)	7 (21.2)	18 (17.0)	5 (15.2)	4 (9.8)	3 (18.0)	57 (15.2)
0.3	14 (18.9)	8 (16.7)	8 (32.0)	5 (15.2)	20 (18.9)	3 (9.1)	7 (17.1)	2 (12.5)	67 (17.8)
0.4	8 (10.8)	6 (12.5)	3 (12.0)	4 (12.1)	11 (10.4)	6 (18.2)	7 (17.1)	1 (6.3)	46 (12.2)
0.5	4 (5.4)	5 (10.4)	1 (4.0)	1 (3.0)	7 (6.6)	2 (6.1)	5 (12.2)	3 (18.8)	28 (7.4)
0.6	6 (8.1)	2 (4.2)	1 (4.0)	2 (6.1)	5 (4.7)	1 (3.0)	3 (7.3)	0 (0.0)	20 (5.3)
0.7	6 (8.1)	4 (8.3)	2 (8.0)	3 (9.1)	3 (2.8)	0 (0.0)	4 (7.8)	0 (0.0)	22 (5.9)
0.8	1 (1.4)	3 (6.3)	0 (0.0))	2 (6.1)	5 (4.7)	2 (6.1)	1 (2.4)	0 (0.0)	14 (3.7)
0.9	3 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.8)	1 (3.0)	0 (0.0)	0 (0.0)	7 (1.9)
1.0	3 (4.1)	2 (4.2)	0 (0.0)	0 (0.0)	6 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	11 (2.9)

Keys: SA: *S. aureus*; SP: *S. pyogenes*; CS: CoN *Staphylococcus* spp; EF: *E. faecalis*; EC: *E coli*; PS: *Proteus* spp PA: *P. aeruginosa*; KP: *K. pneumoniae*, **Values in parenthesis represent percentages**

DISCUSSION

The laboratory screening techniques in specialized areas of medicine have transformed and improved the art of diagnosis (Abdullahi and Thairu, 2015). The MSU specimens are frequently sent for analysis with the intention of reducing morbidity and mortality attributable to UTI. The global prevalence of bacteriuria in pregnancy varies in various studies that have been conducted (Girishbabu, 2011). In our study, the prevalence of bacteriuria among the pregnant women attending antenatal clinics was 30.2 % and this value was higher than 20.0 % reported by Assefa et al. (2008) in Addis Ababa, Ethiopia and lower than 47.5 % reported by Okonko et al. (2009) in Ibadan, Nigeria. The discrepancies in prevalence of bacteriuria among the pregnant women could be attributed to the socioeconomic status, gestational age, lack of personal and environmental hygiene.

In relation to age groups, the highest SBU was among the subjects within ages 21-30 yrs, followed by age group ≤ 20 yrs, 31-40 yrs and the lowest SBU was obtained from the subjects aged ≥ 41 yrs. This finding corroborated the results of Ahmad (2012) who reported the highest SBU (38.1 %) among the subjects between ages 21 and 30 yrs in Kashmir, but differs with Ezeigbo et al (2016) who reported the highest SBU among the subjects within ages 31- 40 yrs. The high SBU among the reproductive age groups especially those within ages ≤ 20 yrs could be ascribed to early marriage and childbearing. The significant outcomes were observed regarding the prevalence of SBU based on gestational periods. The prevalence of SBU based on trimesters in this study was in consistent with the report of Boye et al. (2012) who obtained the highest SBU among the subjects in their second trimester. This result was, however, not in accord with the report by Okonko et al. (2009) who had the highest SBU among the subjects in their third trimester.

The use and reliability of urine nitrite and leukocyte esterase dipstick tests for detection of SBU have been investigated. Of the 245 MSU specimens, 30 (12.2 %) had nitrite (NIT) only, 42 (17.1 %) had leukocyte esterase (LE) only, while 20 (8.2 %) had both NIT and LE. The values obtained for NIT and LE in this study were lower than 21.5 % (NIT) and 30.7 % (LE) reported by Fernandes *et al*. (2018). The MSU cultures of the subjects had *E coli*, *S. aureus*, *S. pyogenes*, *Proteus spp*, *P. aeruginosa*, *K. pneumoniae*, *CoN Staphylococcus spp* and *E. faecalis*. The isolation of these bacteria from the MSU was in harmony with the reports of many researchers (Amadi et al. 2007). In our study, *E. coli* had the highest occurrence (28.2 %) and this finding agreed with the report of Turpin et al. (2007) in Kumasi, Ghana. The high occurrence of E. coli could be due to poor genital hygienic practices by the subjects who may find it difficult to suitably clean their anus/genital after defecating or urinating (Imade et al. 2010).

The varied percentages of slime producing bacterial isolates were obtained from the MSU of the subjects. The occurrence of slime producing S. aureus and E coli in this study agreed with Arslan and Zkardes (2007) and Dadawala et al. (2010) who reported the occurrence of slime S. aureus and E coli in samples using Congo Red Agar. The resistance of the slime producing bacterial isolates to Nitrofurantoin, Ampicillin, Chloramphenicol and Tetracycline were observed in this study and this agreed with Ahmed et al. (2000) who reported the isolation of Ampicillin, Chloramphenicol, Tetracycline and Nitrofurantoin resistant bacterial isolates from the MSU of the subjects in Sudan. Our study also showed the occurrence of MDR bacterial isolates from the MSU specimens of the subjects and this confirmed the findings of Mahroop-Raja and John (2015) and Ekwealor et al. (2016) in Tamil Nadu, India and Awka, Nigeria, respectively.

The occurrence of slime producing MDR **bacterial isolates** in MSU of the subjects in this locality have been established, consequently, there is a need to continuously monitor the antibiotic susceptibility profiles of slime producing bacteria implicated in UTI and also endeavor to avert and/or curtail bacterial slime-associated infections.

REFERENCES

Abdullahi HI, Thairu Y. (2015). Asymptomatic bacteriuria among pregnant women attending antenatal: evaluation of screening test, IOSR Journal Of Pharmacy, 5 (8):41-47.

Ahmad S. (2012). Pattern of urinary tract infection in Kashmir and antimicrobial susceptibility. Bangladesh Medical Research Council Bulletin, 38(.3): 79-83.

Ahmed AA, Osman H, Musa HA, Ahmed AB, Karrar Z. (2000). Antimicrobial agent resistance in bacterial isolates from patients with diarrhoea and UTIs in the Dudan. American Journal of Tropical Medicine and Hygiene , 5: 259 – 263.

Akinjogunla OJ, Adenugba IT, Inyang UO. (2017). Extracellular hydrolytic enzymes and location of multidrug resistance markers in urinary bacterial isolates, World Journal of Biomedical Research, 4 (1): 50-60.

Akinjogunla OJ, Ehinmore I, Atang TD., Akpan I. (2018). Antibacterial efficacy of aqueous leaf extracts of C. citratus (Poaceae) and B. verticillata (Rubiaceae) on biofilm producing uropathogenic bacterial isolates. World Journal of Applied Science and Technology. 10(1b):1-8.

Akyar I, Fidan I, Rota S. (1998). Koagülaznegatif stafilokoklarda slime faktör yapiminin üç farkli yöntemle aras¸tirilmasi, tür tayini ve antibiyotik direnci. Mikrobiyol Bült.; 32:15– 22

Amadi ES, Enemuo OB, Uneke CJ, Nwosu OK, Onyeagba RA, Ugbogu OC. (2007). Asymptomatic bacteriuria among pregnant women in Abakaliki, Ebonyi State, Nigeria. Journal of Medical Sciences, 7(4):698–700.

Amiri M, Lavasani Z, Norouzirad R, Najibpour R, Mohamadpour M, Nikpoor AR, Raeisi M, Marzouni HZ. (2015). Prevalence of UTI among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful City, Iran, 2012 – 2013. Iranian Red Crescent Medical Journal, 17 (8): 1-8.

Ammendolia MG, Di-Rosa R, Montanar OL, Arciolamm CR, Baldassarri L. (1999). Slime production and expression of the slime-associated antigen by staphylococcal clinical isolates. Journal of Clinical Microbiology, 37: 3235-3238.

Arslan S, Zkardes F. (2007). Slime production and antibiotic susceptibility in staphylococci isolated from clinical samples, Mem Inst Oswaldo Cruz, Rio de Janeiro, 102(1): 29-33.

Assefa A, Asrat D, Woldeamanuel Y, Abdella A. (2008). Bacterial profile and drug susceptibility patterns of UTI in pregnant women at Tikur Anbessa specialized ospital Addis Ababa, Ethiopia. Ethiopian Medical Journal, 46(3):227–235.

Barnick CGW, Cardozo LD. (1991). The lower urinary tract in pregnancy, labour and puerperium. In: Studd J, editor. Progress in Obstetrics and Gynaecology Vol. 9.London: Churchill Livingstone, pp 195–204.

Blomberg B, Olsen BE, Hinderaker SG, Langeland N, Gasheka P, Jureen R, Kvale G, Midtvedt T. (2005). Antimicrobial resistance in urinary bacterial isolates from pregnant women in rural Tanzania. Scandinavian Journal of Infectious Diseases, 37: 262-268.

Boye A, Siakwa PM, Boampong JN, Koffuor GA, Amoateng P, Penu G. (2012). Asymptomatic l of urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana, E3 Journal of Medical Research, 1(6): 074-083.

Charles BM. (2011). Urinary tract infections, infectious disease. In: Microbiology and Immunology. University of South Carolina Press. USA. Pp 234-265.

Clinical and Laboratories Standards Institute (2005). Performance Standards for Antimicrobial Susceptibility Testing, 15th informational supplement,; M100-S15. Wayne (PA).

Dadawala AI, Chauhan HC, Chandel BS, Shah NM, Kher HN. (2010). Assessment of E. coli isolates for in vitro biofilm production, Veterinary World, 3(8): 364-366.

Dalzell JE, Lefevre ML. (2000). Urinary tract infection of pregnancy. American Academy of Family Physicians, 61: 713 - 721.

Ekwealor PA, Ugwu MC, Ezeobi I, Amalukwe G, Ugwu BC, Okezie U, Stanley C, Esimon C. (2016). Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. International Journal of Microbiology, 2016: 1-6.

Ezeigbo OR, Nnadozie RIA, Asuoha-Chuks N, Ojiako VU, Awurumv IN, Ugochukwu MG. (2016). Incidence of UTI among pregnant women attending antenatal clinics at some selected hospitals in Aba, Nigeria. International Journal of Current Microbiology and .Applied Science, 5(1): 193-199.

Fernandes DJ, Jaidev MD, Castelino DN. (2018). Utility of dipstick test (nitrite and leukocyte esterase) and microscopic analysis of urine when compared to culture in the diagnosis of UTI in children. International Journal of Contemporary Pediatrics, 5(1):156-160.

Freeman DJ, Falkner FR, Keane CT. (1989). New method for detecting slime production by coagulase negative Staphylococci. Journal of Clinical Pathology, 42:872-874.

Gilstrap LC, Ramin SM. (2001). Urinary tract infections during pregnancy. Obstetric and Gynaecology Clinics North America, 28(3): 581-591.

Girishbabu RJ. (2011). Asymptomatic bacteriuria in pregnancy. International Journal of Biological and Medical Research, 2(3):740-742.

Imade PE, Izekor PE, Eghafona NO, Enabulele OI., Ophori E. (2010). Asymptomatic bacteriuria among pregnant women. North American Journal of Medical Sciences, 2(6): 263–266.

Jan MB, John DT, Sentry A. (2002). High Prevalence of oxacillin-resistant S. aureus isolates from hospitalized patients in Asia-Pacific and South Africa: Results from SENTRY Antimicrobial Surveillance Program, 1998- 1999. Antimicrobial Agents and Chemotherapy, 46(3): 879-888.

Millar LK, Cox SM. (1997). Urinary tract infections complicating pregnancy. Infectious Diseases Clinics of North America, 11: 13-26.

Macejko AM, Schaeffer AJ. (2007). Asymptomatic bacteriuria and symptomatic UTIs during pregnancy.Urologic Clinics of North America, 34: 35–42.

Mahroop RMM, John SA. (2015) Multidrug resistance profile of urinary tract infected gram positive pathogenic bacterial isolates, International Journal of Infections, 2(1):227- 274.

Okonko IO, Ijandipe LA, Ilusanya OA, Donbraye-Emmanuel OB, Ejembi J, Udeze AO (2009). Incidence of urinary tract infection among pregnant women in Ibadan, South -Western Nigeria. African Journal of Biotechnology, 8:6649-6657.

Ratna B, Sharan KM. (2017). Rapid nitrite dip stick vs urine culture for diagnosis of urinary tract infections (UTI): Laboratory prospective. International Journal of Biomedical Research, 8(4): 204-209.

Rupp ME, Archer GD. (1994). Coagulase-negative staphylococci: pathogens associated with medical progress. Clinical Infectious Diseases, 19: 231-245.

Semeniuk H, Church D. (1999). Evaluation of the leukocyte esterase and nitrite urine dipstick screening tests for detection of bacteriuria in women with suspected uncomplicated UTIs. Journal of Clinical Microbiology.37(9):3051-3052.

Turpin CA, Minkah B, Danso KA, Frimpong EH. (2007). Asymptomatic bacteriuria in pregnant women attending antenatal clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. Ghana Medical Journal, 41(1):26–29.

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