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Prevalence and Antibiotic Susceptibility Patterns of Bacteria associated with Urinary Tract Infections among Pregnant Women attending Antenatal Clinic in Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria

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Abstract
Bacterial urinary tract infection is one of the common health problems during pregnancy which can lead to complications. The misuse of antibiotics against the infection has led to emergence of antibiotic resistance bacteria. The aim of the study was to determine the prevalence and antibiotic susceptibility patterns of bacteria associated with urinary tract infections among pregnant women, attending antenatal clinic in Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria. A total of 110clean catch midstream urine (MSU) samples of pregnant women were collected and analyzed from August to November, 2019. The samples were subjected to bacteriological count using spread plate method. Isolation and identification of bacteria were carried out using cultural and biochemical tests. The isolates were subjected to antibiotic susceptibility test using Agar disc diffusion method. The results obtained showed that out of 110 samples collected, 70 (64%) were positive for UTI. Prevalence of UTI was higher among age group <18years (72.7%). Prevalence of UTI based on gestational age showed higher rate in second trimester (71.7%). Prevalence of UTI based on symptoms showed higher rate among women with pain during urination (75.0%) while those with lower back pain had the lowest rate of UTI (50.0%). Prevalence of UTI based on predisposing factors showed higher rate (73.3%) among those that used toilet roll to clean up after defecation than those that used water (62.1%).Those that practice the method of cleaning up from anus to vagina after defecation had higher rate of UTI (68.8%) than those that clean up from vagina to anus (59.7%). However, the rate was not statistically significant (p>0.05). Non-diabetic pregnant women had 63.7% prevalence of UTI while diabetic pregnant women had (62.5%). *Staphylococcus aureus* account for 16.4% of UTI cases in this study area. This was followed by *Escherichia coli* (10.9%), while the least implicated bacteria was *Klebsiella* spp (10%). *Klebsiella* species was 63.6% susceptible and resistant to gentamicin and amoxicillin respectively. *Staphylococcus aureus* was 94.4% susceptible and 44.4% resistance to streptomycin and trimethoprim-sulfamethoxazole respectively. *Escherichia coli* had the highest Multi-Drug Resistant in this study. Due to prevalence of UTI in this study area, it is essential to screen pregnant women for UTI in order to avoid complications during child delivery. Streptomycin was the drug of choice in treating the infection.
Keywords: Pregnancy; Prevalence; Bacterial UTI; Antibiotic Susceptibility; Multi-Drug Resistant

INTRODUCTION

The urinary tract consists of the organs that collect and store urine and release it from the body which include kidneys, bladder and urethra (Ezeugoigwe *et al.*, 2018). Different types of microorganisms are able to invade this tract and cause infection (UTI) (Gomi *et al.*, 2015). It is most often caused by bacteria, but fungi and virus may also be implicated (Amdekar *et al.*, 2011). Gram-negative bacteria cause 90% of bacterial UTI cases while Gram-positive bacteria cause only 10% of the cases. The most frequent isolated bacteria is *Escherichia coli*, accounting for 65%-90% of urinary tract infections (Gupta*et al.*, 2001; Weekes, 2015).

Urinary tract infections have different presentations that range from symptomatic to asymptomatic (Gunes *et al.*, 2005). Asymptomatic bacteriuria is defined as the presence of significant bacteriuria without the symptoms of an acute UTI.Symptomatic UTIs are divided into lower tract (acutecystitis) or upper tract (acute pyelonephritis) infections (Schnarr and Smaill, 2008). Urinary tract infections during pregnancy are among the most common health problems worldwide, especially in developing counties (Dimetry *et al.*, 2007).

In pregnant women, physiological and anatomical changes in the urinary tract, as well as immune system changes during pregnancy increase the prevalence of asymptomatic bacteriuria and in some cases lead to the symptomatic infection, resulting in serious risks for both mother and fetus. The risks include nausea, vomiting, frequent urination, dysuria, premature birth and low birth weight. There may also be cesarean delivery, morphologicalabnormalities and infant mortality (Emamghorashi *et al.*, 2012). There is a significant statistical correlation between UTI and congenital retardation (McDermott, 2000).

Increasing age, parity, diabetes, number of childbirths, number of intercourses per week, sickle cell anemia, history of UTI, urinary tract disorders and immune deficiency may increase the risk of UTI in pregnant women (Counningham *et al.*, 2005; Giraldo *et al.*, 2011; Raza *et al.*, 2011).

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue. Particularly in the developing world where there is high level ofpoverty, illiteracy and poor hygienic practices, there isalso high prevalence of fake and spurious drugs of questionable quality in circulation [Weekes, 2015;Fagan, 2015).The easy availabilityin the community without prescription and low cost make the drugs subject to abuse (Manikandan, 2011).

This study will provide valuable information on the prevalence of the etiology of UTIs in pregnant women and help clinician in using appropriate antibiotics for the management of the infection in the study area.

MATERIALS AND METHODS

Study Areaand Ethical Approval

The study was carried out at antenatal clinic of Ahmadu Bello University Teaching Hospital Shika, Zaria-Nigeria, after obtaining ethical approval from the Hospital Management with NHREC NO. 10/12/2015, D-U-N-S NO.954524802 and ABUTH/HREC/UG/6.

Sample Size, Population and Inclusion Criteria

One hundred and ten (110) consented pregnant women that are attending antenatal clinic were recruited for the study. Urine (Mid-stream) sample was collected from each of the pregnant woman.

Administration of Questionnaire

Standard questionnaires were administered to the pregnant women attending the antenatal clinic to make enquiries on their social-demographic factors, UTI predisposing factors and symptoms.

Samples Collection

Midstream urine samples were aseptically collected from the pregnant women. Clean catch mid-stream urine was collected into a properly labeled, sterile, wide necked, leak proof, plastic universal containers and were processed immediately.

Determination of Bacteriological Count

The collected urine was mixed aseptically, 1ml was transferred with sterile syringe serially into three bottles containing 9ml each of sterile normal saline to make concentration of 10^{-1}, 10^{-2} and 10^{-3}, then 0.1ml from 10^{-3} was aseptically transferred onto already prepared Nutrient Agar Media, evenly spread with sterile bent glass rod and incubated at 37°C aerobically for 24 hours. After the incubation period, colonies on plates were counted using colony counter. Positive UTI were indicated by the presence of 100,000 colony-forming units (CFU) per milliliter in the culture of an appropriately collected MSU (mid-stream urine) (Harding *et al.*, 2002).

Interpretive Criteria

Urine bacteriological count was grouped into two categories. Significant bacteriuria was interpreted as $\geq$100,000 CFU/ml while non-Significant bacteriuria was interpreted as <100,000 CFU/ml.

Isolation of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* Species

A loop full from each urine sample was streaked on already prepared Mannitol Salt Agar, Eosin Methylene Blue Agar and MacConkey Agar plates. The plates were incubated at 37^{0}C for 24 hours. Golden yellow colonies on Mannitol Salt Agar plates were suspected to be *Staphylococcus aureus*, colonies with bluish black and green metallic sheen on Eosin Methylene Blue Agar plates were suspected to be *Escherichia coli* and while those with bright pink and mucoid characteristics on MacConkey agar were suspected to be *Klebsiella* spp. The suspected isolates were aseptically sub-cultured into fresh MSA, EMB and MacConkey agar respectively, then, incubated at 37^{0}C for 24 hours in order to obtain pure isolates. Thereafter, were inoculated into Nutrient Agar Slants, incubated at 37^{0}C for 24 hours and kept in the refrigerator for further analysis.

Biochemical Identification of the Suspected Isolates

Suspected *Staphylococcus aureus* that were positive for Catalase and Coagulase Tests were confirmed to be *Staphylococcus aureus*. Suspected *Escherichia coli* that were positive for Indole and Methyl-Red tests, negative for Voges-Proskauer and Citrate Utilization tests, and with slant and butt to be yellow/yellow without H_2S production but gas production in Triple Sugar Iron utilization test are confirmed to be *Escherichia coli*. The suspected colonies of *Klebsiella* spp. that were positive for Indole, Voges-Proskauer and Citrate Utilization tests but negative for Methyl-Red test, and with slant and butt to be yellow/yellow without H_2S production but gas production in Triple Sugar Iron utilization test are confirmed to be *Klebsiella* spp.

Preparation of MacFarland Standard

McFarland standard was prepared by mixing 0.5ml of 1.175% barium chloride dehydrate ($BaCl_2.2H_2O$ with 9.95ml of 1% sulfuric acid (H_2SO_4) which gives a precipitate of barium sulfate (turbidity) and this turbidity is the reference to which a bacterialsuspension turbidity in normal saline was compared to, to give an approximate bacterial concentration of $1.5x10^8$ CFU/ml (Colony forming unit per ml).

Standardization of Inoculum

To standardize the inoculum, fresh loopful of pure bacteria isolates were suspended into 5ml sterile normal saline and the turbidity compared to that of 0.5 McFarland standards.

Antibiotic Susceptibility Test

Disc agar diffusion method was employed in determining the antibiotic susceptibility of the isolated microorganisms. The following antibiotics discs were used; Chloramphenicol (30µg), Amoxicillin (30µg), Ciprofloxacin (10µg), Gentamicin (30µg), Streptomycin (30µg), Trimethoprim-sulfamethoxazole (30µg), Erythromycin (10µg) and Gentamicin (10µg). A sterile cotton swab was dipped into standardized bacterial isolates suspension, the swab stick was pressedagainst the side of the test tube to get rid of excess inoculum after which the swab stick was swabbed evenly across the entire surface of Mueller Hinton Agar plates and the plates were allowed to dry. Thereafter, antibiotic impregnated discs were placed on the Mueller Hinton Agar plates containing the standardized inoculum using sterile forceps. The discs were placed at least 15mm from the edge of the plate and not closer than 25mm from each other. The discs ware firmly pressed down to ensure their contact with the agar and allowed to diffuse. The plates were incubated at 37^0C for 24 hours, after which zone of inhibitions were measured in millimeter using transparent meter rule. The measurements were interpreted as susceptible, resistant or intermediate using guidelines from Clinical and Laboratory Standards Institute (CLSI) 2018.

RESULTS

Out of 110 urine sample collected from the pregnant women, 70 (63.6%) of the women were found to be UTI positive (Figure 1). Urinary tract infection was found to be higher among age group <18years (72.7%) while age group 18-34 years had the least prevalence (60.8%)(Table 1). The prevalence of UTI was found to be higher in Second trimester (71.7%) and least in Third trimester (57.5%) (Table 2). The prevalence of UTI was found to be highest in pregnant women with pain during urination (75.0%) and least in pregnant women with lower back pain (50.0%)(Table 3).Table 4 shows the prevalence of UTI based on predisposing factors; based onmaterials used to clean up after defecation, pregnant women that used toilet roll after defecation for cleaning up highest UTI rate (73.3%) than those that use water. based on method used to clean up positive after defecation, those that used forward method had the highest rate (68.8%) than those that used backward method. *Staphylococcus aureus* was found to be the highest isolated bacteria (16.4%) in the UTI cases, while *Klebsiella* spp was the least (10%) implicated bacteria (Figure 2).

Table 5 shows percentage of isolates that were susceptible, intermediate and resistance to the antibiotics used. Approximately, 64% of *Klebsiella* isolates were susceptible to each of gentamycin and streptomycin, while 54.5% of them were resistance to chloramphenicol. Sixty seven percent of the *E. coli* isolated was resistance to each of trimethoprim-sulfamethoxazole, chloramphenicol and ciprofloxacin, and also 66.7% to gentamycin. Ninety four percent (94%) of *Staphylococcus aureus* were susceptible to streptomycin, while 44.4% of them were resistance to trimethoprim-sulfamethoxazole. Table6-8shows phenotypic antibiotic susceptibility patterns of the isolated bacteria. Isolates resistance to 3 or more antibiotics were considered to be multi-drug resistant. *Escherichia coli*showed the highest occurrence of Multi-Drug Resistant (MDR) isolates (66.7%), while *Staphylococcus aureus* showed the least occurrence isolates (22.2%) of MDR (Figure 3).

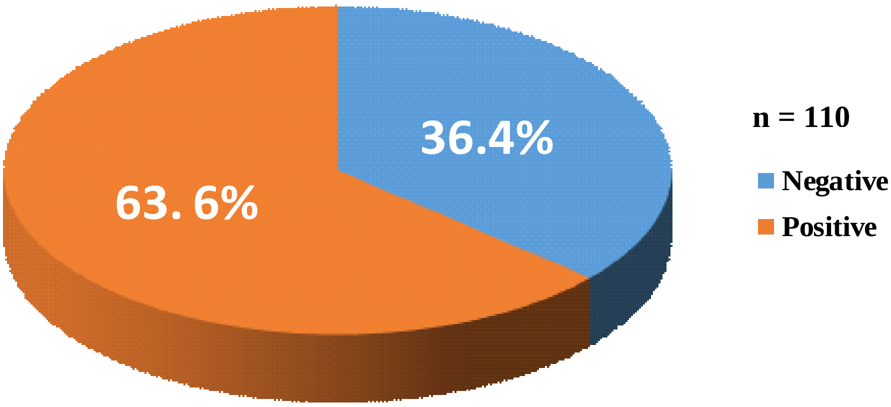

Figure 1: Prevalence of Urinary Tract Infection among Pregnant Women attending ABU Teaching Hospital

Key: n = Number of Sample Collected

Table 1: Prevalence of Urinary Tract Infection Based on Age Groups among Pregnant Women attending ABU Teaching Hospital

Age group (Years)	No. examined	No. positive (%)	No. negative (%)
<18	11	8(72.7)	3(27.3)
18-34	74	45(60.8)	29(39.2)
≥35	25	17(68.0)	8(32.0)
Total	110	70(63.6)	40(36.4)

x^2=0.854; P- value =0.653

Table 2: Prevalence of Urinary Tract Infection Based on Gestation Period among Pregnant Women attending ABU Teaching Hospital

Gestation period	No. examined	No. positive (%)	No. negative (%)
First trimester	24	14(58.3)	10(41.7)
Second trimester	46	33(71.7)	13(28.3)
Third trimester	40	23(57.5)	17(42.5)
Total	110	70(63.6)	40(36.4)

x^2=2.248; P-value=0.325

Table 3: Prevalence of Urinary Tract Infection Based on Symptoms among Pregnant Women attending ABU Teaching Hospital

Symptoms	Number examined	Number positive (%)	Number negative (%)	x^2	P-value
Abnormal vaginal discharge					
Yes	26	17(65.4)	9(34.6)	0.45	0.832
No	84	53(63.1)	31(36.9)		
Abdominal pain					
Yes	37	24(64.9)	13(35.1)		
No	73	46(63.0)	27(37.0)	0.36	0.849
Pain during urination	12	9(75.0)	3(25.0)		
Yes	98	61(62.2)	37(37.8)	0.752	0.386
No					
Lower back pain					
Yes	16	8(50.0)	8(50.0)		
No	94	62(66.0)	32(34.0)	1.505	0.220

Table 4: Prevalence of Urinary Tract Infection Based on Predisposing Factors among Pregnant Women attending ABU Teaching Hospital

Predisposing factors	Number examined	Number positive (%)	Number negative (%)	x^2P-value
Material used to clean up after defecation				
Toilet roll	15	11(73.3)	4(26.7)	0.7060.401
Water	95	59(62.1)	36(37.9)	
Method used to clean up				
Anus to vagina	48	33(68.8)	15(31.2)	0.9620.327
Vagina to anus	62	37(59.7)	25(40.3)	
Are you diabetic				
Yes	8	5(62.5)	3(37.5)	0.945 *0.005
No	102	65(63.7)	37(36.3)	

Key: *Statistically Associated

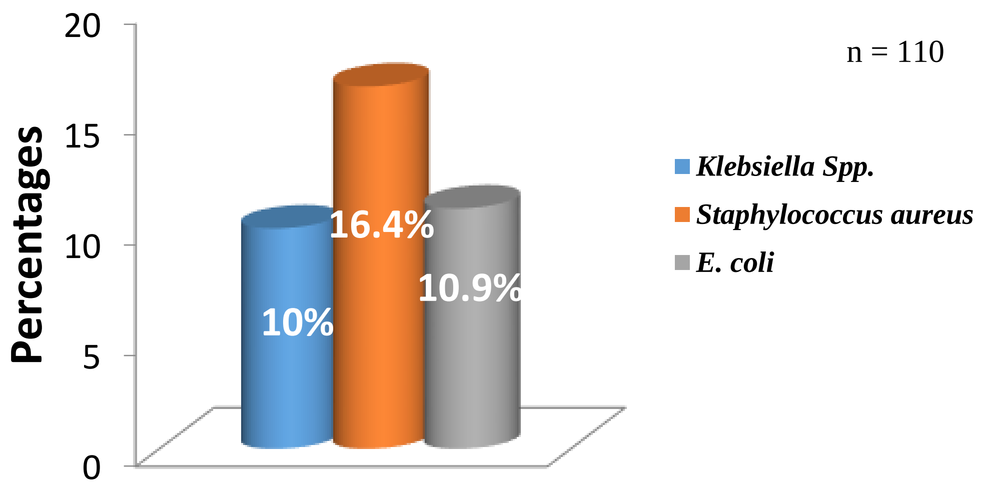

Figure 2: Prevalence of Bacteria Associated with Urinary Tract Infections among Pregnant Women attending ABU Teaching Hospital
Key: n = Number of Sample Collected

Table 5: Antibiotic Susceptibility Profile of Bacteria Associated with Urinary Tract Infections among Pregnant Women attending ABU Teaching Hospital

	Klebsiella spp			*Escherichia coli*			*Staphylococcus aureus*		
	S	I	R	S	I	R	S	I	R
Antibiotics		n (%)			n (%)			n (%)	
SXT	-	-	-	4(33.3)	0(0)	8(66.7)	9(50)	1(5.6)	8(44.4)
CH	4(36.4)	1(9.1)	6(54.5)	4(33.3)	0(0)	8(66.7)	-	-	-
CPX	6(54.5)	0(0)	5(45.5)	4(33.3)	0(0)	8(66.7)	13(72.2)	1(5.6)	4(22.2)
CN	7(63.6)	0(0)	4(36.4)	8(66.7)	0(0)	4(33.3)	14(77.8)	1(5.6)	3(16.7)
S	7(63.6)	0(0)	4(36.4)	6(50)	1(8.3)	5(41.7)	17(94.4)	0(0)	1(5.6)
E	-	-	-	-	-	-	8(44.4)	4(22.2)	6(33.3)
AM	3(27.3)	1(9.1)	7(63.6)	-	-	-	-	-	-

Table 7: Phenotypic Antibiotic Susceptibility Patterns of *Escherichia coli*

Isolate code	Susceptible	Intermediate	Resistance	Multi-Drug Resistant(MDR)
PE1			SXT,CH,CPX,CN,S	+
PE6			SXT,CH,CPX,CN,S	+
PE8	SXT,CPX,CN,S		CH	-
PE14	CN,S		CPX,CH,SXT	+
PE15			SXT,CH,CN,CPX,S	+
PE23	CN,S		SXT,CH,CPX	+
PE24	CN	S	CPX,CH,SXT	+
PE25	S		CN,CH,CPX,SXT	+
PE73	CH,CPX,CN,S,SXT			-
PE92	CH,CPX,CN,SXT		S	-
PE99	CH,CN		CPX,S,SXT	+
PE102	S,CH,CN,CPX,SXT			-

Key: R=Resistance; I=Intermediate; S=Susceptible; CH=Chloramphenicol; SXT=Trimethoprim-sulfamethoxazole; CPX=Ciprofloxacin; CN=Gentamycin; S=Streptomycin;

Table 8: Phenotypic Antibiotic Susceptibility Patterns of *Staphylococcus aureus*

Isolate code	Susceptible	Intermediate	Resistance	Multi-Drug Resistant(MDR)
PS1	SXT,E,CPX,CN,S			-
PS2	SXT,CPX,CN,S	E		-
PS6	CN,S		SXT,CPX,E	+
PS7	CPX,CN,S	SXT	E	-
PS11	CN,S,E		SXT,CPX	-
PS34	CN,S,CPX,SXT		E	-
PS37	CN,S,E		SXT,CPX	-
PS38	CN,S,CPX,SXT	E		-
PS73	S	CN	CPX,SXT,E	+
PS74	CPX,CN,S	E	SXT	-
PS75	S,CPX,CN,SXT	E		-
PS77	E,CPX,S		SXT,CN	-
PS78	S	CPX	SXT,CN,E	+
PS79	CPX		SXT,CN,E,S	+
PS83	SXT,E,CPX,CN,S			-
PS87	SXT,E,CPX,CN,S			-
PS100	SXT,E,CPX,CN,S			-
PS101	SXT,E,CPX,CN,S			-

Key: R=Resistance; I=Intermediate; S=Susceptible; E=Erythromycin; SXT= Trimethoprim- sulfamethoxazole; CPX=Ciprofloxacin; CN=Gentamycin; S=Streptomycin;

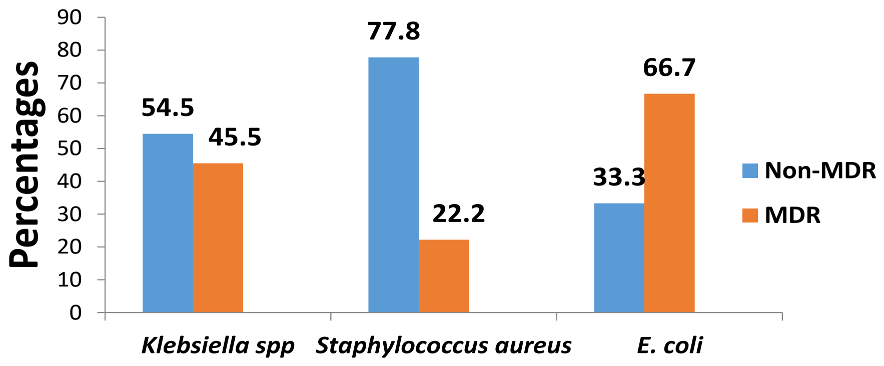

Figure 3: Occurrence of Multi-Drug Resistant Bacteria Isolated from Urinary Tract Infections

DISCUSSION

In this study, the prevalence of UTI was 64%. This rate is higher than the 31.6% recorded in Kano (Jido *et al.*, 2006) and 14.6% in Mwanza, Tanzania (Masinde *et al.*, 2009). The higher rate in our study could be due to the higher number of pregnant women sampled. It may also be due to poor personal hygiene, difference in social-demographic factors, predisposing factors and alteration in vaginal microflora which encourages vaginal colonization with coliforms and this can result to UTI (Kolawale *et al.*, 2009).

Prevalence of UTI was higher among age group <18 years (72.7%) followed by ≥35 years (68.0%) and least in 18-34 years (60.8%). These findings were in contrast to the one recorded against age group 26-30 years (31.7%) in Abuja, Nigeria by Yunusa *et al.,* (2015). The difference could be as a result of early teenage pregnancy or may be as a result of higher sexual activities among this age group which in turn increased UTI risk. However, there was no association between the age groups and UTI (p>0.05).

Prevalence of UTI was higher among pregnant women at 2ndtrimester (71.7%), followed by those at 1st trimester (58.3%) and least was among pregnant women at the 3rd trimester (57.5%). This findings was in contrast to the one recorded in Abuja by Yunusa *et al.,* (2015), where UTI was higher in 1sttrimester (48.%) than other trimesters, 3rd trimester (44.2%) and 2nd trimester (37.7%). The difference may be due to the fact that most of the pregnant women in our study area came for antenatal care during Second trimester.

Prevalence of UTI was higher among pregnant women with abnormal vaginal discharge (65.4%) than those without abnormal vaginal discharge (63.1%). However, it was not statistically significant (p> 0.05).This findings was in contrast to the one recorded in Maiduguri, Nigeria by Sanusi *et al.*, (2016), where prevalence was (31.5%) in those without abnormal vaginal discharge.

Prevalence of UTI was higher among pregnant women with abdominal pain (64.9%) than those without abdominal pain (63.0%). However, there was no statistical significant (p> 0.05).

Prevalence of UTI was higher in pregnant women that used toilet roll to clean up after defecation (73.3%) than those that used water (62.1%). This could be as a result of the fact that there would be remain of feaces when toilet roll is used. However, there was no statistical association between material of cleaning up and the infection.

Prevalence of UTI was higher in pregnant women that clean up from anus to vagina after defecation (68.8%) than those that cleaned up from vagina to anus (59.7%). This was nearly similar with the one observed by Hala *et al.* (2016) in Menoufia Governorate, where UTI prevalence was (50.7%) among those that cleaned up from vagina to anus.

Prevalence of UTI was higher in pregnant women without diabetes (63.7%) than those with diabetes (62.5%). However, it's not statistically significant (p> 0.05).This findings was in contrast to Majeda *et al.*, (2016) where the prevalence was (10.9%) among non-diabetes pregnant women in Oman. This difference could be due to higher number of pregnant women without diabetes in the study.

Staphylococcus aureus was more encountered among pregnant women with UTI (16.4%), this was followed by *Esherichia coli* (10.9%). The least encountered bacterium was *Klebsiella* spp with 10% occurrence.

This trend is similar to the findings of Sanusi *et al.*, (2016) in terms of how higher their occurrence is in Maiduguri, where *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species were 9%, 5% and 2% respectively. However, these findings were in contrast to several studies conducted which showed that *Escherichia coli* were more encountered in UTI than *Staphylococcus aureus* (Mohammad, 2013; Masinde *et al.*, 2009; Dimetry *et al.*, 2007). Presence of members of Enterobacteriaceae family such as *Escherichia coli, Klebsiella spp* means that the infection was as a result of an increase in levels of amino acids and lactose during pregnancy that particularly encourages *Escherichia coli* growth. It could also be due to infection by fecal contamination due to poor hygiene (Obiogbolu *et al.*, 2009). Nworie and Eze, (2010), attributed the high prevalence of Staphylococcal infection to poor hygiene.

Klebsiella species was 63.6% susceptible and resistance to gentamicin and amoxicillin respectively. The percentage susceptibility was similar to that of Sanusi*et al.*, (2016) in Maiduguri, Nigeria, where he reported that *Klebsiella*specieswere64% susceptible to gentamicin and the resistance to amoxicillin was in contrast where there was 10.5% resistance of *Klebsiella* species to amoxicillin.

Escherichia coli were 66.7% susceptible and resistance to gentamicin and chloramphenicol respectively. The susceptibility outcome of *E. coli* was nearly similar to that of Sanusi *et al.*, (2016) in Maiduguri, where they were 65% susceptible to gentamicin (65%).

Staphylococcus aureus was 94.4% susceptible and 44.4% resistance to streptomycin and trimethoprim-sulfamethoxazole respectively. This was in contrast to Onoh *et al*., (2013) in Abakaliki, where the organism was 50% susceptible to streptomycin.
Escherichia coli had the highest multi-drug resistant isolates in this study, likewise as reported to have higher resistance towards different antimicrobials in Latin American and Costa Rica, respectively (Gales *et al.,* 2002; Williams *et al*., 2003).

CONCLUSION

Urinary tract infection is the most common cause of admission in Obstetrics wards. The prevalence of UTI in this study was 64%. *Staphylococcus aureus* had the highest occurrence rate (16.4%) followed by *Escherichia coli* (10.9%) and *Klebsiella* spp (10%).

RECOMMENDATION

Pregnant women and general public should be advised to use water for cleanup instead of toilet roll after defecation to minimize rate of exposure to UTI. Pregnant women should be advised to clean up from vagina to anus after defecation to minimize exposure to UTI. Health talks on preventive measures of the diseases associated with pregnancy should be often provided. Specific guidelines should be set for testing antimicrobial susceptibility.Early treatment reduces the likelihood of complications and costs of patient care.

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