Original Research Article

Prevalence of Uropathogens and Associated Risk Factors in Urinary Tract Infection in Pregnant Women Attending Antenatal Clinic in a Teaching Hospital in Awka, Nigeria

ABSTRACT

Urinary tract infection (UTI) is one of the common problems encountered in pregnancy, and it is associated with significant maternal and fetal risks. The prevalence of UTI varies worldwide. This study was undertaken to determine the prevalence of uropathogens and associated risk factors in UTI in pregnant women in Awka, Nigeria. This was a cross sectional study that involved 269 pregnant women. Clean-catch mid-stream morning urine specimens were collected and examined macroscopically, microscopically and bacteriologically. Information on Socio-demographic characteristics and some clinical factors were collected using pre-designed questionnaires. Statistical analysis was done using SPSS version 22 and the p-value was set at P=0.05. A total of 118 isolates were obtained and the prevalence of UTI in the study population was 39.8%. The most predominant bacterium was *Enterococcus faecalis* 34(29%), followed by *Escherichia coli* 21(17.8%), *Klebsiella pneumoniae* 15(12.7%), *Staphylococcus aureus* 10(8.4%), *Staphyloccocus saprophyticus* 7(6.0%), *Enterobacter cloacae* 6(5.1%), and *Staphyloccocus epidermidis* 4(3.4%). *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Proteus mirabilis* occurred at frequency of 3(2.5%) each while *Pseudomonas oryzihabitans* and *Morganella morganii* occurred at 2(1.7%) each. *Candida* species were also isolated, including *Candida albicans* 4(3.4%), *Candida krusei*3(2.5%) and *Candida guilliermondii*1(0.8%). None of the socio-demographic variables were statistically associated with UTI. Teachings on personal hygiene during every antenatal visit can help prevent UTI in pregnant women.

Key words: Uropathogens, Mid-stream urine, Antenatal, Socio-demographic variables

1. INTRODUCTION

Urinary tract infections (UTIs) are infections that result when microbial count of culture of midstream urine specimens is greater than 10^5cfu/ml [1]; with or without clinical symptoms such as dysuria, fever, urgency, flank pain and hematuria [2, 3, 4]. [5] stated that these high counts which are fairly constant in serial specimens from the same patient, reflect bacteria multiplication in the urine *in vivo* and are accepted as indicating significant bacteriuria. UTI could be both community and hospital acquired infections [6]. Community acquired urinary tract infection (CA-UTI); occurs in the community or within less than 48 hours of hospital admission and was not incubating at the time of hospital admission [7]. Hospital acquired UTI (nosocomial UTI) could also be defined as the onset of UTI in patients, 48 hours after admission [2].

UTI is one of the common bacterial infections that complicate pregnancy [8] hence the reason for choice of pregnant women as the study group in this research. In pregnancy it may be symptomatic, commonly manifested as urethritis, cystitis or pyelonephritis; or it may remain asymptomatic [9]. Pregnancy however, enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to other adverse obstetric outcomes such as prematurity, low-birth weight and higher fetal mortality rates [1].The significance of UTI in pregnancy has been widely evaluated at several studies from different countries such as Ethiopia, Ghana, and Nigeria [10, 11, 9, 12].

In Nigeria, the prevalence of UTI among antenatal patients has been reported in Benin, Ebonyi and Nassarawa States as 13.8%, 55% and 62.67% respectively [13, 14, 15]. The predisposing determinants of high prevalence of UTI in pregnancy include induced ureteral dilation, urinary stasis, reduced immune function, and presence of vesicoureteric reflex and difficulty with hygiene due to a distended pregnant belly [9, 15, 8]. It has also been reported that up to 70% of pregnant women develop glycosuria, which encourages bacteria growth in urine [10, 1].

Uropathogens which are organisms that cause UTI are those from the normal vaginal, perineal and fecal flora [9]. They include *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Proteus*

mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa,Citrobacter species, *Streptococcus agalactiae* and *Candida* species [16, 8, 2]. The predominant organisms that cause UTIs during pregnancy are *E. coli* which accounts for 80%-90% of infection [1, 9].

There is paucity of data on the prevalence of UTI and its associated risk factors in Awka, the capital City of Anambra State. Although a study has been carried out in Awka metropolis, reporting on the bacteriological aetiology and incidence of UTIs among pregnant women [17], the associated risk factors were not evaluated. This study was therefore aimed to determine the prevalence, uropathogens and associated risk factors of both asymptomatic and symptomatic UTIs in antenatal patients at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra State, Nigeria. It serves as a referral center for Hospitals in Anambra State and beyond. The analysis of specimens collected was done at Medical Laboratory Department of Regina Caeli Hospital and Maternity, Awka. Further confirmation of isolates was done at Evans Biomedics (Nig), Clinical and Analytical Laboratory, Oyigbo; Rivers State, Nigeria.

2.2 Study design

The study with reference number- (COOUTH/AA/VOI.1.010) was approved by the ethical committee of the hospital (COOUTH). This study was a cross sectional study which was carried out over a period of nine months from 11th of March to 31st December, 2016.

2.3 Study population

The study population comprised of pregnant women attending antenatal clinic (ANC) as outpatients at COOUTH. The study participants included those women who have not taken antibiotics within 2 weeks before presentation at the clinic and also were willing to participate in the study. Slovin's (1960) formula (modified by [18]) was used to calculate the appropriate sample size from the population which was calculated as 269 participants. The 269 pregnant women were randomly selected on presentation at ANC. Socio-demographic variables (age, marital status, education level, residence/address and other clinical information such as parity, gravidity, trimester, history of catheterization, and history of UTI) were obtained from the patients using pre-designed questionnaires.

2.4 Specimen collection and analysis

Clean-catch midstream (morning) urine were used for the study. Sterile leak-proof specimen bottles (which have been previously labeled) were distributed to the women after they were instructed on how to collect such specimens by the matron in charge of ANC (which involves the initial cleaning of the urethral area with clean water) and the importance of clean catch midstream urine in the study. The specimens received from patients were transported to the laboratory in specimen box containing ice pack [19]and laboratory analysis undertaken immediately within 1-2 hours of collection [4]. The urine specimens were examined macroscopically for colour and turbidity as described by [20] and [7]. Microscopically, specimens were analyzed using wet preparation to detect presence of pus cells (i.e white blood cells), red cells, casts, parasites, yeast cells, crystals, bacterial cells (since the urine were freshly collected) as described by [4] and [21].

The specimens were cultured on Cystine Lactose Electrolyte Deficient (Rapid Labs) media with a loop that holds an inoculum of 0.002ml and incubated aerobically at 37°C for 18–24 hours.Following incubation, significant bacterial growth was taken as any count of uniform colonies equal to or in excess of 10^4 CFU per milliliter of urine [22, 23]. The colony count/bacterial numbers were estimated using a simple Mathematical method [4]. Isolated organisms were identified based on their colony morphology, Gram- stain reactions and biochemical tests including motility, catalase, coagulase, sugar fermentation, urease, oxidase, Triple Sugar Iron (TSI), citrate utilization, indole, litmus milk decolorization, and Eosin Methylene Blue (EMB) agar tests. The bacterial isolates were further

confirmed by culturing in Chromagar Orientation. The fungal isolates, in addition to direct microscopy of wet unstained preparation and germ tube test were confirmed to species level using Chromogenic Candida Agar (CCA). Polymicrobial growth was considered significant only after complete evaluation of clinical symptoms of UTI, history of UTI, the patient's age, catheterization, pus cells, and bacterial count [24].

2.5 Statistical Analysis

Data were coded, entered and analyzed using Statistical Package for Social Science (SPSS), version 22, EPI Info® version 7.2.1.0. Study findings were explained in words and tables. Results/ proportions for categorical variables were compared using percentages, 2x2 tables and chi square test. In all cases, P-value ≤ 0.05 was taken as statistically significant.

3. RESULTS

Out of the 269 specimens examined for UTI, 107 showed significant microbial growth giving an overall prevalence of 39.8%. The result of macroscopy and microscopy urinalysis has been reported [20]. Of all considered risk factors, none was statistically associated with UTI. However, greater prevalence of UTI was observed among those women within the age group of 16-20 years (50%),rural dwellers (41%), no formal education (55.6%) and with history of catheterization (43%) (Table1). Gram positive, Gram negative bacteria including *Candida* species were identified in this study (Tables 2, 3 and 4). A total of 118 isolates were obtained out of 107 specimens that yielded significant microbial growth (Table 5). Some specimens showed polymicrobial growth 11(4.09%), non-significant andno growth 40(14.87) {Fig.1}. Of all the isolates, bacteria were more prevalent 110(93.2%) than yeast 8(6.8%) which were *Candida* species. Gram-positive and Gram-negative bacteria occurred at equal proportion in this study 55(45.6%); though the enterobacteria had the highest prevalence of 84(71.2%) compared to other isolates 34(28.8%).

Table 1: Prevalence of UTI in relation to socio-demographics and clinical variables of study population at COOUTH

Variables	Description	No tested (%)	Negative UTI No(%)	Positive UTI No (%)	*P*- value
Age (years)	16-20	4(1.5)	2(50)	2(50)	0.943
	21-25	52(19.3)	30(58)	22(42)	
	26-30	96(35.7)	57(59.4)	39(40.6)	
	31-35	85(31.6)	52(61)	33(39)	
	>35	32(11.9)	21(66)	11(34)	
Marital status	Married	266(99)	160(60)	106(40)	0.652
	Other	3(1)	2(66.7)	1(33.3)	
Residence	Urban	171(64)	104(60.8)	67(39.2)	0.446
	Rural	98(36)	58(59)	40(41)	
Educational status	None	9(3.3)	4(44.4)	5(55.6)	0.391
	Primary	4(1.5)	3(75)	1(25)	
	Secondary	72(26.8)	39(54.2)	33(45.8)	
	Tertiary	184(68.4)	116(63)	68(37)	
Symptoms of UTI	Yes	48(18)	28(58)	20(42)	0.445
	No	221(82)	134(61)	87(39)	

Gravidity	1-3	232(86)	138(59.5)	94(40.5)	0.746
	4-6	35(13)	23(65.7)	12 (34.3)	
	7-9	2(1)	1(50)	1(50)	
Parity	Nulliparous	82(30.4)	49(60)	33(40)	0.992
	Monoparous	103(38.3)	62(60.2)	41(39.8)	
	Multiparous	84(31.2)	51(60.7)	33(39.3)	
Trimester	1st	22(8.2)	13(59.1)	9(40.9)	0.976
	2nd	90(33.4)	55(61)	35(39)	
	3rd	157(58.4)	94(60)	63(40)	
History of catheterization	Yes	35(13)	20(57)	15(43)	0.412
	No	234(87)	142(60.7)	92(39.3)	
History of UTI	Yes	58(22)	39(67)	19(33)	0.139
	No	211(78)	123(58.3)	88(41.7)	

Table 2: Morphological and Biochemical Characterization of Gram Positive Bacterial Isolates

S/ No	Colony Characteristics on CLED medium	Gram Reaction	Catalase	Coagulase	Sucrose	Glucose	Lactose	Fructose	Maltose	Mannitol	Litmus milk medium	Growth on Blood agar	Colony Appearnce on Chromagar Orientation	Isolate Identified as
1.	Deep yellow colonies	Positive cocci in clusters	+	+	+	+	+	+	+	+	NT	Cream with β-hemolysis	Golden and opaque	*Staphylococcus aureus*
2.	Yellow to white colonies	Positive cocci in clusters	+	-	+	+	+	+	+	+	NT	NT	Pink, opaque and small	*Staphylococcus saprophyticus*
3.	Whitish discrete colonies	Positive cocci in clusters	+	-	+	+	+	+	+	-	NT	Small, whitish with β-	Clear and translucent	*Staphylococcus epidermidis*

												hemolysis		
4.	Small yellow colonies	Positive cocci in pairs and short chains	-	-	+	+	+	+	+	+	+	White with β-hemolysis	Torquoise blue	*Enterococcus faecalis*

Fermentation tests:

+ = Sugar fermented with acid production

- = Sugar not fermented

+ = Positive

- = Negative

NT = Not tested

Table 3: Morphological and Biochemical Characteristics of Gram Negative Isolates

S/No	Appearance on CLED Media	Appearance on EMB Agar	Motility	Indole	Urease	Simmon citrate	Oxidase	Sucrose	Fructose	Maltose	Lactose	Manitol	Glucose	TSIA				Appearance on Macconkey Agar	Growth on Blood Agar	Appearance on Chromagar Orientation	Isolate Identified as:
														Slope	Butt	H_2S	Gas				
1.	Yellow opaque colonies with deeper colouredc entre	Blue-black colonies with green metallic sheen	+	+	-	-	-	d	+	+	+	+	+	Y	Y	-	d	Pinkish colonies	NT	Dark pink to reddish	*Escherichia coli*
2.	Large mucoid yellow or yellow-white colonies	Dark purple colonies	-	-	+	+	-	+	+	+	+	+	+	Y	Y	-	+	Pinkish and large	NT	Dark metallic blue with or without brown halo	*Klebsiella pneumoniae*
3.	Blue-grey colonies	Colourless colonies	+	-	+	-	-	-	-	-	-	-	+	R	Y	+	+	Colourless colonies	NT	Clear with or without brown halo	*Proteus mirabilis*

4.	Green colonies with rough periphery	Colourless colonies	+	-	-	-	+	-	-	-	-	-	-	R	R	-	-	Colourless colonies	Light pink with dark green pigmentation	Cream and translucent	*Pseudomonas aeruginosa*
5.	Yellowish-white, mucoid/ moist colonies	Pinkish colonies	+	-	-	D	-	+	+	+	+	+	+	Y	Y	-	+	Pinkish/ brick red colonies		Dark/metallic blue	*Enterobacter cloacae*
6.	Translucent or colourless opaque-grey colonies and moist colonies	Colourless colonies	+	+	+	+	-	+	+	+	-	+	+	Y	Y	-	+	Colourless colonies	Large,whitish with α(partial) hemolysis	Violet purple interior with cream burder	*Citrobacter freundii*
7.	Opaque/grey discrete colonies	Colourless colonies	+	+	+	-	-	-	+	+	-	-	+	R	Y	-	d	Colourless colonies	NT	Clear with brown halo	*Morganella morganii*
8.	Green colonies	Purple/ grey colonies	+	-	-	-	-	-	-	-	-	-	+	R	Y	-	-	Wrinkled pinkish colonies with serated edges	NT	Cream and translucent	*Pseudomonas oryzihabitans*

S= Serial, CLED = Cystine Lactose Electrolyte Deficient, R = Red-pink (alkaline reaction), Y = Yellow (Acid reaction)
EMB = Eosin Methylene Blue agar, d = different strains give different reaction, TSI = Tripple sugar iron agar, NT= Not tested

H_2S = Hydrogen sulphide, + = Positive, - = Negative

Table 4: Morphological and Biochemical Characteristics of *Candida* Isolates

S/No	Appearance on CLED Agar	Gram reaction	Fermentation						Growth on Sabouraud's Dextrose Agar (SDA)	Growth on Chromogenic Candida Agar (CCA)	*Candida* Isolate
			Glucose	Maltose	Sucrose	Lactose	Fructose	Germ tube			
1.	Small whitish and moist colonies	Positive cells of *Candida*	+	+	-	-	+	+	Small, whitish and moist	Light green coloured colonies	*Candida albicans*
2.	Small whitish and moist	Positive cells of *Candida*	+	-	-	-	+	-	Small, whitish and moist	Light pink coloured colonies	*Candida guilliermondii*
3.	Small whitish and moist	Positive cells of *Candida*	+	-	-	-	+	-	Small, whitish and moist	Purple fuzzy colonies with spreading pale edges	*Candida krusei*

CLED = Cystine Lactose Electrolyte Deficient
\+ = Positive
\- = Negative

Table 5: Frequency of isolates

Isolated uropathogen	**No.**	(%)
Enterococcus faecalis	34	29.0
Escherichia coli	21	17.8
Klebsiella pneumonia	15	12.7
Staphylococcus aureus	10	8.4
Staphylococcus saprophyticus	7	6.0
Enterobacter cloacae	6	5.1
Staphylococcus epidermidis	4	3.4
Citrobacter fruendii	3	2.5
Proteus mirabilis	3	2.5
Pseudomonas aeruginosa	3	2.5
Pseudomonas oryzihabitans	2	1.7
Morganella morganii	2	1.7
Candida albicans	4	3.4
Candida krusei	3	2.5
Candida guilliermondii	1	0.8
TOTAL	**118**	**100**

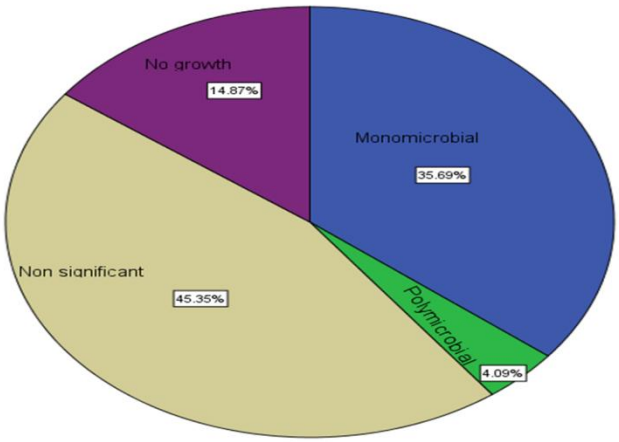

Figure 1: Characteristic growth pattern of urine culture among antenatal patients at COOUTH

4. DISCUSSIONS

The overall prevalence of urinary tract infection in this study was 39.8% which was found concordant with 39%, 49.4% observed in two different locations (Mehdipatnam and Kanataka)in

India [8, 25]; 40% and 47.5% observed in Ilorin and Ibadan both in Nigeria [26, 27] among the same study group. Lower prevalence of 10.1% and 21% was reported by [2] at Ghana and [28] at Benin, Nigeria. Higher prevalence (55% and 62.67%) compared to that observed in this study was also reported in Nigeria at Afikpo, Ebonyi State and Karu, Nasarawa State respectively [14, 15]. These variations may be explained by the fact that there are differences in geographical locations, social habits of the community, drainage system, standard of personal hygiene and education [27, 10, 25].

Bacterial count as demonstrated in polymicrobial infection in this study was up to 10^4cfu/ml and this is in line with the findings of [22]. Our result 11(10.3%) of polymicrobial growth is consistent with that of [24] (9.7%) but different from that of [22] who reported polymicrobial prevalence of 2.26% in their study findings.

The frequency of isolates in this study revealed a changing trend in the bacterial profile found in bacteriuria among women attending antenatal clinic. From most previous studies apart from [11] that reported that *Enterococcus faecalis* 4(26.7%) was predominantly isolated among pregnant women in Ghana, it has been very uncommon to recover *Enterococcus faecalis* as the most prevalent isolate as seen in this work. Most findings from other studies have reported *E. coli* as the most frequently isolated pathogen both in Nigeria, Ethiopia, Ghana and Iran [9, 10, 2, 23]. Enterococci are hardy, facultatively anaerobic Gram positive cocci in pairs or short chains that can grow and survive in many environments [29]. This organism has been noted as a significant bacterial isolate from women with UTI in pregnancy [9]. This study has recorded the predominance of enterobacteria compared to other isolates and this could be explained by the fact that they are intestinal normal flora, which might enter the urethra through poor personal hygiene such as washing or cleaning perineum (incorrectly) from back to front.The equal proportion of Gram positive and Gram negative bacteria observed in this study was because Gram positive cocci were the most predominant. With the exception of *Enterococcus faecalis*, the Gram negative bacteria were the most frequently isolated organisms. The high prevalence of Gram negative enterobacteria could be as a result of unique structures which aid the attachment to the uroepithelial cells and prevent bacteria from urinary bladder lavage, allowing them to invade the tissues and multiply giving rise to invasive infection and pyelonephritis in pregnancy [9]. Secondly this dominance observed in Gram negative enterobacteria could also be attributed to an increase in levels of amino acids and lactose during pregnancy which particularly encourages *E. coli* growth [3].*Pseudomonas oryzihabitans*2(1.7%) isolated in this study has been reported as a rare pathogen from clinical specimens [33] though it has been incriminated in some studies by [31] who reported 11 cases of *P. oryzihabitans* in hospital setting in China, while [32] and [33] reported a case each of UTI caused by *P. oryzihabitans*in Turkey and India respectively. *Candida* species with the prevalence of 8(6.7%) isolated in this study in addition to numerous bacteria was in agreement with the study at India [24] that observed *Candida* species (9.3%); in addition to bacteria isolated in their study. A lower prevalence (2.5%) was reported in the same scenario by [27]. *Candida* being a normal flora in the vagina and perineum, it could be understood that under favorable humid condition, and by a certain way of multiplication and

ascending of the agent to urethral meatus, infection can be initiated at this point [30]. Candiduria observed in this study could also be as a result of the higher incidence of vaginal candidiasis in pregnant women when compared to the non -pregnant subjects [16].

Urinary tract infection affects all age groups and both sexes [27]. The higher prevalence of UTI among younger women in this study which decreased steadily among the older women could be as a result of increased sexual activity usually observed in younger than older women [15]. However this study didn't capture the sexual activity within the subjects which is one of the limitations of the study. The higher prevalence of positive urine cultures which was observed among women from rural areas 40(41%) could be that the women were not enlightened and not aware of the need for proper personal hygiene. The highest prevalence of positive urine cultures among those with no formal education 5(55%) could be attributed to lack of awareness/poor knowledge of practicing personal hygiene in pregnancy and in agreement with a study at Nnewi, Nigeria [16].The higher prevalence of UTI (though not statistically significant) among those with history of catheterization is in agreement with findings of [19]; among pregnant women at Felege Hiwet Referral Hospital, Bahir Dar, North West Ethiopia. Other studies that reported a statistically significant relationship between the use of catheter and UTI prevalence include [1] and [10]. The non-significant relationship observed in this study could be because in normal clinical practice, introduction of catheter is usually done aseptically and the patients were normally placed on antibiotics throughout the period of catheterization. Also the reason could be because the period of hospitalization was not determined in this study. Pregnant women with history of UTI had a lower prevalence of UTI, 19(33%) in this study. This finding is in agreement with the study at Ethiopia [10] that also reported a lower prevalence of 13(6.4%) among those with UTI history. This lower prevalence observed in this study could be as a result of effective treatment of the previous infection, which ruled out the possibility of any resistant strains. However, the relationship between history and prevalence of UTI as observed in this study was not statistically significant. This finding however varied from those of other researchers [1, 19, 30] who reported higher prevalence of UTI among those with previous history of UTI.

All the risk factors of UTI evaluated in this work were associated with a higher prevalence of UTI but none was statistically significant which is in agreement with studies by [9] and [30] among pregnant women at Federal Medical center and Ebonyi State University Teaching Hospital Abakaliki Ebonyi State, Nigeria and Abha General Hospital Saudi Arabia. There is a possibility that perhaps UTI among these women were predisposed by other associated risk factors of UTI including maternal anaemia, poor personal hygiene, previous use of contraceptives, and increased sexual activity during pregnancy among others as observed by [30] and [19]. These associated risk factors should therefore be critically examined in further research studies.

5. CONCLUSION

The overall prevalence of UTI in this study is 39.8%. *Enterococcus faecalis* was the most frequently isolated bacterium from urine of pregnant women attending antenatal clinic at COOUTH Awka. From this study, it was also discovered that *Pseudomonas oryzihabitans* was incriminated in urinary tract infection (UTI) in pregnancy. This pathogen has not been reported to cause urinary tract infection in Nigeria. Previous research findings have reported only

Candida albicans as aetiologic agents of UTI, but from our study it was obvious that non-*Candida albicans* species (*C. krusei*and *C. guilliermondii*) were now involved as causative agents of UTI in pregnancy in the study area. There was no significant statistical association between the prevalence of UTI and other factors like maternal age, symptoms of UTI, address/residence, educational and marital status, gravidity, parity, trimester, history of catheterization and UTI. Considering the high prevalence in this study, it is vital to introduce screening of pregnant women for UTI as part of antenatal care.

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