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Prevalence of Uropathogenic Escherichia coli Among Pregnant Women at Adeoyo Maternity Hospital, Ibadan, Oyo State

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Abstract
The study assessed the prevalence of Uropathogenic Escherichia coli (UPEC) and related factors isolated from the urine samples of pregnant women seeking antenatal care at Adeoyo maternity clinic. UPEC have been noted to be the most common bacterial infection reported in pregnancy. Pregnant women are more predisposed to UTI due to hormonal and physiological changes in the urinary tract including ureteral dilatation and changes in bladders volume and tone. A total of 218 Fresh void Midstream urine samples collected from the participants were analyzed using standard microbiological methods. Isolates were identified by standard biochemical methods while a well-structured questionnaire was used to collect data from the participants on cofactors. Out of the 218 urine samples analyzed, (45.4%) had significant bacteriuria indicating presence of UTI. Among those that had positive bacteriuria, UPEC accounted for 9.3%, while Klebsiella (20.4%), Staphylococcus (8.3%), Candida (6.9%), Proteus (0.9%), and Enterococcus (0.5%). Parity was found to be significantly associated with bacteriuria (p value = 0.019), while patients' age, gestational age, occupation, marital status, level of education and history of UTI did not have any significant association with UTI (p value >0.05). The study concluded Uropathogenic Escherichia coli as the second most predominant pathogen after Klebsiella species. It further showed that parity was significantly associated with UTI in pregnancy, it is therefore important to regularly carryout routine surveillance and monitoring to update clinicians on the prevalent pathogens and their associated risk factors which may be a guide in rational and empirical UTI treatment in pregnancy.

Keywords: Bacteriuria, Prevalence, Urinary Tract Infection, Uropathogenic, E. coli**.**

Introduction

Escherichia coli (E. coli) is a normal flora of both human and animal gastrointestinal tract (GIT). It usually forms a symbiotic association with its host and plays important role in maintaining normal homeostasis in the GIT, while promoting the stability of the luminal flora. (Yan &Paik, 2004). *Escherichia coli* are usually confined to the intestinal lumen and rarely cause disease, disease condition however can arise when the gastrointestinal barriers are breached in debilitated or immunosuppressed host. Some strains of *E. coli* can however diverge into a pathogenic strain, through the acquisition of new genes, either by horizontal gene transfer of transposon, plasmid, bacteriophage and pathogenicity island or by mutation, which enhances their ability to adapt to new niches and cause diseases.

Pathogenic strains of *E. coli* have been broadly classified into two groups namely: Enteric/diarrheagenic pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) according to Kaper et al. (2004.). Extra intestinal pathogenic *Escherichia coli,* which includes Uropathogenic *E. coli* (UPEC), maintains the ability to exist in the gut without causing any disease but has the ability to disseminate and colonize other host niches including the blood, central nervous system and urinary tract resulting in diseases. (Wiles et al.,2008).

Uropathogeneic *E. coli* (UPEC**)** infections are the most common hospital and community acquired infection worldwide (Espinar et al., 2015). UPEC are *E. coli* with specific virulence factor that are capable of colonizing the

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periureteral area, and enter the urinary tract to cause disease. Furthermore, UPEC remains the predominant (70-90%) uropathogen isolated from infections in anatomically normal, unobstructed urinary tracts and is also responsible for 85% of asymptomatic bacteriuria and more than 65% cystitis. According to the study reported by Foxman (2003). Moreso, UPEC urinary tract infection is the most common infection reported in pregnancy (Dwyer & O'RreIlly, 2007). Pregnant women are more predisposed to UPEC UTI due to hormonal and physiological changes in the urinary tract including ureteral dilatation and changes in bladders volume and tone (Nowicki, 2002). The incidence of UTI during pregnancy is higher among women who have had childhood infections than those without such history (Martinell et al., 1990). Moreso, the obstruction of urine outflow by the conceiving uterus has been used to demonstrate the mechanism of UTI in pregnant women (Gabbe et al.,2007). This supposition however, does not regard the receptor etiology of the ascending UTI or gestational alteration of the immunity. Furthermore, UTI can be dangerous for both the mother and the fetus, Preterm delivery, increased incidence of intra uterine growth restriction and to a lesser extend pre-eclampsia, caesarean delivery, anemia, sepsis and septic shock are complications that can arise from UTI during pregnancy (Mazor et al., 2009). Therefore, the aim of this study was to determine the prevalence of UPEC in UTI among pregnant women at Adeoyo Marternity Clinic to guide the choice of rational and empirical treatment in pregnancy.

Aim and Objectives of the Study.

The study was aimed to determine the prevalence of UPEC UTI in pregnancy among the study population. Specific Objectives are:

1. To determine the prevalence of UPEC in UTI among pregnant women at Adeoyo Maternity Clinic.
2. To investigate the factors associated with UPEC UTI in pregnancy among the study group.

Materials and Methods

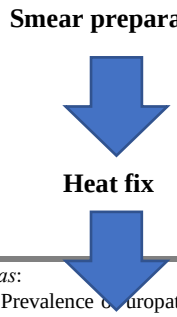
Sample Collection and Bacteria Identification

Fresh void mid-stream urine samples were collected from consenting 218 pregnant women at Adeoyo Maternity clinic. The urine sample was analyzed microscopically for the present of pus cells, crystals, cast, epithelial cells, yeast cells, red blood cells etc. thereafter the urine were inoculated on standard bacteriological media (Blood and MacConkey agar) using a standardize wire loop to deliver 0.1ml of the urine into the agar plate. The inoculated agar plates were incubated at 37^{o}C for 24hours. All isolates with significant bacteriuria indicated by the presence of up to 100,000CFU/ml, were identified using colonial morphology, Gram stain and biochemical tests. All UPEC isolates were identified using ATCC 117755 as a control specimen for the procedure.

Biochemical Test.

Gram stain

A drop of normal saline was placed on a grease free slide, a colony of the organism was transferred unto the glass slide using a sterile wire loop. A thin smear was made and allowed to air-dry in a safe place, the slide was thereafter heat fixed by passing thrice over the flame. The slide was thereafter stained using Gram staining reagents including: crystal violet which is the primary stain, lugos iodine a mordant to fix the primary stain on the cell, 95% ethanol as decolorizing agent and safranine as counter stain. The slides were thereafter examined microscopically using oil immersion objectives. Isolates which were pink and rod shaped where subjected to biochemical tests for further identification. A known *E. coli* and *S. aurens* stained slides were used as Gram negative and positive control.



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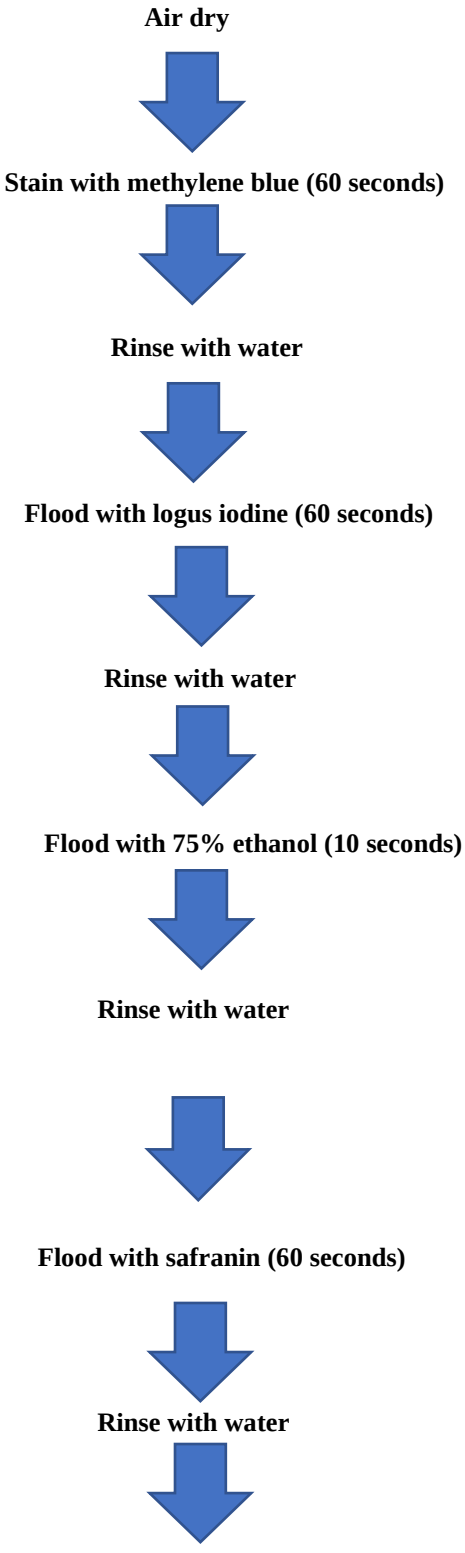

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Indole Test

This was performed on isolates which appeared as Gram negative bacilli from the Gram stain. The isolates were inoculated in a bijou bottle containing 3ml of sterile tryptone water and was incubated at 37°C for 24-48hours, 0.5ml of Kovac reagent was added to the suspension and shake gently and thereafter observed for brick-red color on the surface area. Those that showed red color were documented as positive while others were recorded negative. Control organisms used were: *E. coli* as positive control, and *S. aurens* as negative control.

Citrate Utilization Test.

This was used to different between *E. coli* and indole producing *Klebsiella* species. All gram-negative bacilli with suspected *E. coli* morphology were subjected to this test. Isolates were suspended in saline and inoculated into Simon's citrate agar slant using a straight wire loop to make a streak first and then stabbed into the medium. The slant was incubated at 37°C for 24-48hours and was there after observed for a bright blue color in the medium which indicated citrate utilization and hence positive test, while those that retained the green color of the medium were negative. Controls: *Klebsiella* was used as positive control while *E. coli* was used as a negative control. More over biochemical tests such as methyl red, motility, catalase, coagulase, oxidase, and germ tube test etc. were employed for the identification of other bacteria isolated from the urine sample. Each isolate was further confirmed using the criteria on Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and protocols by Cheesbrough. (2010).

Statistical Analysis

Data obtained from the study was sorted and entered in spreadsheet while analysis was done using International Business Machine- Statistical Package for the Social Sciences (IBM*SPSS, USA Chicago, IL version 23.0 for windows). Descriptive statistics of frequency, percentage, charts, mean±SD were used to summarize and present the results. Chi-square test of independence was employed to investigate the relationship between categorical variables while student t-test was employed to compare means of two groups. Results were rated statistically significant if $p<0.05$.

Results

A total of two hundred and eighteen (218) samples were collected and processed during this study. Out of the 218 samples 98(45.4%) had significant bacteriuria and culture positive, while 118(54.6%) were negative for culture. Among the culture positive isolates, UPEC accounted for 20(9.3%) of the UTI causes while 78 (36.1%) were due to *Klebsiella, Candida, Staphylococcus, Proteus,* and *Enterococcus* species, this shows that UPEC has low prevalence (9.3% out of 45%) among the study group as seen in Table 1.

Table 2: Shows the Association of Socio-Demographic factors with UPEC UTI. It showed that there was a statistical association between parity and UPEC UTI ($X^2 = 13.526$, P-Value = 0.019.**)**, it showed the highest percentage of UTI occurrence by parity as 11.6%, followed by 10.7% and the lowest percentage incidence of UPEC UTI by parity is 4.5%. indicating that multiple pregnancies increases the chance of UTI.

It further showed association of UPEC UTI with age. The study revealed that pregnant women between the ages of 23-27 had higher incidence 8(11.9%), followed by ages between 28-32 years 6(8.6%). While pregnant women within the age of 45 and above had the least chance of UPEC UTI but there was no significant association between the UTI and the age of participants. The table went further to show the association of UPEC UIT with gestational age, where it was shown that women in the second and third trimesters (20 -29 weeks) of their pregnancies had the highest prevalence of 16% and 12.2% respectively while women in the early month of their pregnancy had no specific bacteria growth and shows no sign of UTIs. This shows that the incidence of UTIs among pregnant women could also be contributed by gestational age although this study didn't record any significant association between the two variables. Furthermore, the table revealed that UPEC UTI appeared to be more prevalent among self-employed women who constituted 10.9% of the pregnant women with UPEC UTIs, followed by the employed (6.5%) again there was no significant association between the two variables ($P<0.05$).

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Table 3: Shows the association of Medical History with UPEC UTI. Among all pregnant women in the study, 98 (31.3%) reported with symptom suggestive of an UTI and reproductive tract infection (RTI), including abnormal discharge (40.3%), and vaginal itch (45.4%) and previous history of UTI (8.2%). Among those women reported with symptoms, 23 (23.3%) had significant UPEC growth in urine culture while 35 (72.9%) of pregnant women without such symptoms also gave a positive UPEC urine culture. There was no significant association between UPEC UTI and previous medical history.

Table 1: Prevalence of UPEC UTI

UPEC UTI	Frequency	Percent
Positive	20	9.3
Negative	198	90.7
Total	218	100.0

Table 2: Associations of Socio-Demographic Characteristics and UPEC

Attributes	**No Tested n (%)**	**UPEC + n (%)**	**UPEC- n (%)**	**X^2**	**P-Value**
Age (years)					
18-22	14(6.5)		14(100)		
23-27	67(31)	8(11.9)	59(88.1)		
28-32	70(32.4)	6(8.6)	64(91.4)	7.43	0.191
33-37	41(19)	2(4.9)	39(95.1)		
38-42	22(10.2)	3(13.6)	19(86.4)		
≥43	2(0.9)	1(50)	1(50)		
Total		20(9.3)	196(90.7)		
Gestational Age (years)					
≤10			2(100)		
11-14					

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15-19	216(100)		2(100)		
20-24			4(100)	3.157	0.189
25-29		4(16)	21(84)		
30-34		6(12.2)	43(87.8)		
35-39	2(0.9)	7(7.6)	85(92.4)		
Total	2(0.9)	3(7.3)	38(92.7)		
Parity		20(9.3)	195(90.7)		
1	4(1.9)				
2		11(11.6)	84(88.4)		
3	25(11.6)	6(10.7)	50(89.3)		
4	49(22.8)	1(2.6)	37(97.4)	13.526	0.019*
5		1(4.5)	21(95.5)		
6	92(42.8)		4(100)		
Total	41(19.1)	1(100)	0(0)		
Education		20(9.3)	196(90.7)		
Tertiary	215(100)				
Secondary		11(9.3)			
Primary	95(44)	9(9.4)	107(90.7)		
Total			87(90.6)	0.206	0.902
Occupation	56(25.9)	20(9.3)	2(100)		
Employed			196(90.7)		
Self-Employed	38(17.6)	3(6.5)			
Unemployed	22(10.2)	17(10.9)		2.337	0.311
Total			43(93.5)		
	4(1.9)		139(89.1)		
	1(0.5)	20(9.3)			
			14(100)		
	216(100)		196(90.7)		
	118(54.6)				

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	96(44.4)				
	2(0.9)				
	216(100)				
	46(21.3)				
	156(72.2)				
	14(6.5)				
	216(100)				

*** significant at p<0.05, X^2: Chi-square**

TABEL 3: Associations of UPEC UTI WITH Medical History.

Attributes	**No Tested n (%)**	**UPEC + n (%)**	**UPEC- n (%)**	**X^2**	**P-Value**
Vagina Itching					
Yes	98(45.4)	7(7.1)	91(72.9)		
No	118(54.6)	13(11)	105(89)	0.956	0.328
Total	216(100)	20(9.3)	196(90.7)		
Abnormal Vagina Discharge					
Yes	87(40.3)	7(8)	80(92)		
No	129(59.7)	13(10.1)	116(89.9)	0.255	
Total	216(100)	20(9.3)	196(90.7)		0.613
UTI History					
Yes	110(8.2)	9(8.2)	101(91.8)		
No	106(10.4)	11(10.4)	95(89.6)	0.310	
Total	216(100)	20(9.3)	196(90.7)		

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Last UTI Treatment (Months)					0.578
No Treatment	124(58.8)	14(11.3)	110(88.7)		
1-3	15(7.1)		15(100)	4.586	
4-6	14(6.6)		14(100)		
7-12	25(11.8)	1(4)	24(96)		
>12	33(15.6)	3(9.1)	30(90.9)		0.333
Total	211(100)	18(8.5)	193(91.5)		

*** significant at p<0.05, X^2: Chi-square**

Discussion

The prevalence of UPEC UTI in this study population was found to be 9.3%. This shows that UPEC was the second most prevalent bacterial pathogen isolated from the study group, after *Klesiella* species with the prevalence rate of 21.8%. This is in agreement with the study reported in south -western Uganda by Johnson et al., (2021) that *Klebsiella pneumoniae* (37.41%) was the most prevalent uropathogen among pregnant women while UPEC (28.78%) was reported as the second most prevalent. The study also is in alignment with a study reported Vinod & Selvaraj. (2012) which reported K. pneumoniae (65%) as the most prevalent uropthogen. The findings of this study is however different from the reports in few studies where UPEC pathogen were reported to be the most frequently associated with UTIs, few of those studies included Yeva et al., 2020, Charles et al., (2021); Simon-Oke & Odeyemi (2019); & Nwachukwu et al.,(2018). which reported prevalence of UPEC to be 26.7%, 59%, 31.7%, and 47.2%, respectively. The low prevalence recorded in this study could be attributed to seasonal variation as incidence of UTI reduces drastically in dry season. Moreover, the quality of healthcare services and treatment could also be a major contributing factor while the hygiene of participants in addition to sampling method could also account for the desperation in the results.
Different factors have been reported to be associated with UPEC UTI among pregnant women. These included previous UTI history, age, parity, gestational age, occupation, and level of education.

In this study, it was found that pregnant women who reported with symptom suggestive of an UTI and reproductive tract infection (RTI), including abnormal discharge (40.3%), and vaginal itch (45.4%) and previous history of UTI (8.2%). Had low incidence while the incidence of UPEC UTI was high among asymptomatic group (72.9%) indicated by a positive UPEC urine culture. The use of over the counter (OTC) antibiotics, insertions and topical cream, or other traditional medicine to cub symptoms may have accounted for the low incidence in symptomatic participants observed in this study. There was however no significant association between UPEC UTI and previous medical history. This is in line with the studies reported by Hamdan et al. (2017) on UTI in Sudan, and Kavavisarach et al. (2009) whose study on UTI in Thailand revealed absent of significant association between UTI and Previous UTI history. Furthermore, Age was reported in this study to have no significant association (p>0.05) with UPEC UTI, although pregnant women between the ages of 23-27 had higher incidence (11.9%), followed by ages between 28-32 years (8.6%). While pregnant women within the age of 45 and above had the least chance of UPEC UTI. this shows that the risk of UTI declined with increase in age, this could be as a result of body mastery and improved hygiene of the older group. The finding in this study is in agreement with that of Kovavisarach et al. (2009). This study further revealed that there was no significant association between gestational age and UPEC UTI. But it was however observed in this study that women in the second and third trimesters (20 -29 weeks) of their pregnancies had the highest prevalence of 16% and 12.2% respectively while women in the early month of their pregnancy had no specific bacteria growth and shows no sign of UTIs. This shows that the incidence of UPEC UTI among pregnant women could also be contributed by gestational age. This could be as a result of anatomical and physiological changes such as ureteral dilation, urinary stasis and decreased bladder capacity at the said age of pregnancy. This is in line with the report from other studies such as Hamdan et al. (2017).

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More so, the study revealed that UPEC UTI appeared to be more prevalent among self-employed women who constituted 10.9% of the pregnant women with UPEC UTIs, followed by the employed (6.5%). Frequent use of shared/public lavatories by both the self-employed and employed groups could account for the result revealed in this study. Again, there was no significant association between the two variables ($P<0.05$). Parity was revealed in this study to be significantly associated with UPEC UTI (p value = 0.019), This could be so due to the increased dilation of the genitals as a result of multiple pregnancies and in most cases poor aseptic delivery procedure and postpartum UTI management. It is therefore, penitent to constantly carryout routine UTI surveillance in pregnancy to rule out risk factors and complications which may arise as a result of either unidentified or untreated UTI during pregnancy. this is in agreement with the report of Sheikh et al., (2000) and also similar to those recorded by (Gilstrap et al., 2001; & Dimetry et al., 2007) and many other studies. It is however different from few other studies including Masinde et al. (2009), Hazhir et al. (2007) & Turpin et al. (2007) who reported absence of statistically significant association between the two variables.
The differences in the study regarding the statistical association of the proposed risk factors with UPEC UTI among different studies may be due to differences in sampling styles, study population (hygiene level and authenticity of information provided) and the sizes used for each study.

Conclusion

Uropathogenic Escherichia coli was the second most predominant pathogen in the study after Klebsiella species. It is therefore important to regularly carryout routine surveillance and monitoring to update clinicians on the prevalent pathogens, this may be a guide in rational and empirical UTI treatment in pregnancy.

Recommendation

A shift in prevalence will definitely affect the antibiogram pattern and treatment efficiency. It is therefore recommended that a regular routine surveillance and monitoring of both the prevalent pathogen and its antibiotic susceptibility pattern should be carried out to update clinicians on the prevalent pathogens and the appropriate antibiotics therapy for both rational and empirical UTI treatment in pregnancy.

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