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Isolation and Antimicrobial Susceptibility Profile of *Escherichia coli* from Urine Samples of Pregnant Women Attending Antenatal Clinics in Selected Hospitals within Kaduna Metropolis, Nigeria

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Abstract

Urinary tract infections (UTIs) remains the most common infections diagnosed among in and out patients as well as hospitalized patients. A total of 400 urine samples were collected from pregnant women attending selected hospitals within Kaduna Metropolis, Nigeria and analyzed for *E. coli* associated urinary tract infection. *E. coli* was isolated using Eosin methylene blue agar, and preserved on Nutrient agar slant. The isolates were identified based on cultural characteristics, conventional biochemical test and microgen kit identification. The antimicrobial susceptibility pattern of the isolate was determined using the Kirby-Bauer disc diffusion method. Out of the 400 samples collected, 85 (21.3%) were positive for *Escherichia coli*. In relation to the age-group, pregnant women within the age group 26-30 years had the highest prevalence of infection (28.3%), followed by pregnant women within the age of 31-35 years (18.9%), pregnant women with the least prevalence are within the age 15-20 years (18.3%). Pregnant women who were housewives had the highest prevalence of *E. coli* infection. Women in their 2nd and 3rd trimesters were found to have a higher prevalence of *Escherichia coli* infection; 21.7% and 23.0% respectively. This shows that high parity is one of the possible factors affecting the prevalence of urinary tract infection among pregnant women. *Escherichia coli* isolates showed highest susceptibility to Gentamicin (72.9%), Amoxycillin (70.5%) and Streptomycin (70.5%). However, the isolates showed higher resistance to Perfloracin (49.4%), Chloramphenicol (38.8%), and Ciprofloxacin (36.5%).

Keywords: Urinary Trait Infection, Pregnant women, *Escherichia coli*, antimicrobial susceptibility profile

INTRODUCTION

Urinary tract infections (UTIs) represent one of the most common diseases encountered in medical practice today (Aiyegoro *et al.*, 2007). UTI is a predominant type of bacterial infection among pregnant women (Nowicki *et al.*, 2002). *Escherichia coli*, *Escherichia faecalis*, *Klesiella pneumonia*, *Staphylococcus aureus* and *Proteus mirabilis* are most common bacteria causing UTI in human. *Escherichia coli* accounts for approximately 85% of community acquired UTIs and 50% (Ramanath and Shafiya, 2011). Different factors like age, gender, immunosuppression and urological instruments may affect prevalence of UTIs (Iqbal *et al.*, 2010). Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population (Okafor and Elenwo, 2008). About 150 million individuals have been reported to be affected by UTIs annually worldwide (Ntonifor and Ajayi, 2007). Urinary tract infections occur as a result of the microbial colonization of urine and the invasion of any structure of the urinary tract by microbial organisms such as bacteria, viruses, yeasts and parasites (Okafor and Elenwo, 2008).

Urinary Tract Infection is more common in females than in males due to the fact that the urethra is shorter in women. The lower third of the urethra is continually contaminated with pathogens from the vagina and the rectum. In addition, women tend not to empty their bladders as completely as men do. The female urogenital system is exposed to bacteria during intercourse (Raisa and Edward, 2019). Female urethra structurally is found to be less effective for preventing the bacterial entry (Ebief *et al.*, 2001; Kolawole *et al.*, 2009). It may be due to the proximity of the genital tract and urethra and adherence of urothelial mucosa to the mucopolysaccharide lining (Schaeffer *et al.*, 2001; Akortha *et al.*, 2008). The other main factors which make females more prone to UTI are pregnancy and sexual activity (Arul *et al.*, 2012). In pregnancy, the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine (Friedman, 2007).

Also in the non pregnant state the uterus is situated over the bladder whereas in the pregnant state the enlarged uterus affects the urinary tract (Friedman 2013). Sexual activity in females also increases the risk of urethra contamination as the bacteria could be pushed into the urethra during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during child birth (Ebie *et al.*, 2001; Kolawole *et al.*, 2009). Asymptomatic UTI in particular has been associated with an increased risk of developing pyelonephritis, maternal and infant morbidity, pre-term labour and low birth weight (Oyagade *et al.*, 2004).

Hormonal and physiological changes in bladder volume and tone, may promote infection in pregnant women (Nowicki *et al.*, 2002). About 150 million individuals have been reported to be affected by UTIs annually worldwide (Ntonifor and Ajayi, 2007). UTIs of both bacterial and parasitic origins have been associated with high incidence of squamous cell carcinoma of the bladder and the cervix (Schwartz, 1984).

It is the second most prevalent tropical disease after malaria (Lengeler *et al.*, 2002) most of the cases of UTI occur as community acquired infection (Akram *et al.*, 2007).

In Ghana, the prevalence of this disease ranges between 54.8% and 60.0% and this is an indication that the disease is still a major problem in some part of the country (Anto, 2013).

The purpose of this study is to isolate *Escherichia coli* from urine samples of pregnant women attending antenatal in selected hospitals within Kaduna, Nigeria and to determine its susceptibility profile to some selected antibiotics.

MATERIALS AND METHODS

Study Area

This study was carried out in some selected hospitals within Kaduna metropolis Nigeria, which includes: Barau Dikko Teaching Hospital, 44 Nigerian Army Reference Hospital Tudun Wada, Yusuf Dantsoho Memorial Hospital Tudun Wada, General Hospital Kawo, General Hospital Sabon Tasha and Gwamna Awan General Hospital Nassarawa.

Collection of urine samples

A total of 400 samples were collected with the assistance of the laboratory staff of the selected Hospitals. Midstream urine was collected into 20ml calibrated sterile screw-capped universal containers distributed to the pregnant women. The specimens were labeled appropriately and transported to the laboratory for analysis.

Isolation of *E. coli*

The turbid urine samples were streaked immediately on MacConkey agar and it was incubated at 37°C for 24 hours. The suspected colonies which were smooth pinkish colonies were picked and subcultured on Eosine methylene blue agar using a flamed wireloop and it was incubated at 37°C for 24 hours (Paramesh *et al.*, 2018).

The plate with the positive growth shows a greenish metallic sheen. Nutrient agar medium was prepared to preserve pure isolates. The nutrient agar slant was preserved in the refrigerator for further use.

With a sterile wire loop, isolates were Gram stained. This was done by flaming the wire loop and allowing it to cool. A drop of sterile water was placed on a clean glass slide, the sterile loop was used to pick a colony into the drop of distilled water to make a smear. It was air dried, heat fixed, then stained with crystal violet for 1 min. It was rinsed off with distilled water and stained with Lugol's iodine for 1 min, after which it was rinsed with distilled water and flooded with 95% ethanol until no purple colour runs off. It was rinsed again with distilled water, and lastly stained with Safranin for 1 min. It was rinsed with water, blot dried with tissue paper, then examined under oil immersion lens x100 objectives (Cheesbrough, 2006).

Biochemical Characterization of *E. coli*

Motility Test:

The motility medium was prepared by dissolving the required amount of nutrient agar powder in distilled water (according to manufacturer's instruction) and autoclaved. The medium was inoculated by making a fine stab with an inoculating needle to a depth of 1-2cm short of the bottom of the tube. This was then incubated at 37°C for 24 to 48 hours. Motility of an organism is indicated by a line of inoculation which is not sharply defined and the rest of the medium would be cloudy. If the organism is not motile, growth would be restricted to the line of inoculation which becomes sharply defined (Varun, 2016).

Methyl Red- Voges Proskauer Test (MR-VP):

The MR-VP broth was prepared by weighing the specified quantity of the MR-VP agar in distilled water and it was autoclaved. About 5ml of MR-VP broth was inoculated with the organism and incubated for 48-72 hours at 35°C. After incubation, 1ml of the broth was transferred to a small serological tube. About 2-3 drops of methyl red were added to it. A red colour on addition of the indicator signifies a positive methyl red test. While a yellow colour signifies a negative test. To the rest of the broth in the original tube, 5 drops of 40% potassium hydroxide (KOH) were added, followed by 15

drops of 5% α -naphthol in ethanol. The tubes were shaken with the cap loosened and placed in a slanted position. The development of a red colour starting from the liquid-air interface within 1 hour indicates a VP positive test. Where there is no colour change indicates a negative test (Varun, 2016).

Citrate Utilization Test

The specified quantity of the simon citrate powder was weighed and dissolved in distilled water. It was swirled to mix and autoclaved, after which the tube was slanted for the agar to solidify. The agar slant was inoculated with the organism and incubated for 24 to 72 hours. The development of a deep blue colour indicates a positive test (Varun, 2016).

Carbohydrate Fermentation Test in Triple Sugar Iron (TSI) Agar

With a sterile needle, the organism to be tested was picked and streaked over the surface of the slant and the butt was stabbed 2 or 3 times by dipping the sterile needle down to the bottom of the tube. The cap of the tube was closed loosely and incubated at 35°C for 24 hours (Varun, 2016).

Urease Test

Each of the isolated colonies was grown in peptone water and incubated at 37°C for 24 hours. Each of the prepared urea agar slant was inoculated with the peptone water broth and then incubated at 37°C for 24-48 hours. The absence of a bright pink colour after incubation signifies a negative test. While a positive test is indicated by a bright pink colour (Varun, 2016).

Confirmation of *E. coli* using Microgen™ GnA-ID Kit

A colony was emulsified from an 18-24 hour culture in 3ml sterile 0.9% saline for the GnA microwell test strips. The adhesive tape sealing the microwell strips were carefully removed. Three to four drops of the bacterial suspension were added to each well of the mineral oil. The top of the microwell test strips were read after 18-24 hours incubation. The adhesive tape was removed and all positive reactions were recorded with the aid of the colourful chart. Two drops of Kovac's reagent were added to well 8 and read after 60 seconds. One drop of each of VP I and VP II reagent were added to well 10 and read after 15-30 minutes. One drop of TDA reagent was added to well 12 and read after 60 seconds. The adhesive tape was removed and all reactions read and recorded according to manufacturer's instructions. *E. coli* was identified using the Microgen kit software which involves the imputation of the octal code generated.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was performed on the isolates to determine their susceptibility patterns to particular antibiotics. Ten antibiotics were used which includes Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Perfloracin, Tarvid and Streptomycin (Oxoid Ltd, UK). This test was performed using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 2002). Standard inocula of 0.5 McFarland standard were prepared. Mueller Hinton agar was prepared according to manufacturer instruction and was poured into plates and was allowed to solidify. The Mueller Hinton agar plates were inoculated with the standard of inocula using sterile swab sticks. The antibiotic disc was picked using a sterile forceps and was placed on the inoculated agar plate, allowed for diffusion and then incubated at 37°C for 24hrs. Clear zone of growth inhibited was measured and interpreted as either Resistant (R), Intermediate (I) or Susceptible (S) using the Clinical and Laboratory Standard Institute (CLSI) guide lines (CLSI, 2018).

RESULTS

Figure 1 represents the prevalence of *E. coli* among pregnant women in selected hospitals within Kaduna metropolis. Out of the 400 urine samples examined, only eighty-five, 85 (21.3%) samples were positive for *Escherichia coli*.

In this study, the pregnant women examined were classified into the different age groups. The result obtained revealed no significant association between age and incidence of *Escherichia coli* infection ($\chi^2=7.331$, $df=4$, $p=0.119$). A higher prevalence of 28.3% *Escherichia coli* was recorded among subjects within the age group of 26-30, while those in age group 36-40 had the least prevalence of 11.5%.

The data obtained with respect to toilet type in relation to prevalence of *Escherichia coli* showed a significant difference ($\chi^2=74.084$, $df=2$, $p=0.0$). Highest prevalence of (43.0%) was recorded among pregnant women that use pit toilet and the least prevalence of (7.0%) was recorded among pregnant women that use water cistern.

There was statistically significant association between the prevalence of *Escherichia coli* and the socio-economic status of the study population examined ($\chi^2=12.891$, $df=3$, $p=0.005^*$). The highest prevalence of (31.4%) was recorded among pregnant women who are unemployed (House wives). The least prevalence of (14.0%) was observed among the pregnant civil servant.

The prevalence *E.coli* infection in relation to parity showed no significant difference ($\chi^2=36.868$, df =2, p=0.0*). The highest prevalence of (41.9%) was recorded among pregnant women in their third pregnancy. While the least prevalence of (12.4%) was recorded among pregnant women in their second pregnancy. The prevalence of *E.coli* infection in relation to trimester showed a significant difference ($\chi^2=0.961$, df = 2, p= 0.618). The highest prevalence of (23.0%) was recorded among pregnant women in their 3rd trimester of pregnancy. The least prevalence of (16.7%) was observed among pregnant women in their first trimester.

Discharges as a symptom in relation to *Escherichia coli* showed no significant association ($\chi^2=0.462$, df=5, p=0.530). Higher prevalence of (23.0%) was found in pregnant women who do not have discharges when compared with the prevalence of (20.2%) in pregnant women who have discharges. There is a significant association between Abdominal

pain and the prevalence of *E.coli* ($\chi^2=0.023$, df= d p=0.903). The highest prevalence of (21.6%) was recorded among pregnant women without abdominal pain compared to 20.9% who have abdominal pain. Fever as a symptoms in relation to *Escherichia coli* infection revealed a significant association ($\chi^2=3.946$, df=5, p=0.047)

Itchy sensation as a symptom in relation to *Escherichia coli* infection revealed a significant association ($\chi^2=11.588$, df=5, p=0.001). Higher prevalence of (36.1%) was recorded among pregnant women vomiting/spitting compared to the prevalence of 15.4% observed among those not vomiting/spitting.

Table 6 shows the antimicrobial susceptibility pattern of *Escherichia coli* from urine sample. The *Escherichia coli* isolates showed highest susceptibility to Gentamicin (72.9%), Amoxycillin (70.5%) and Streptomycin (70.5%). However, the isolates showed higher resistance to Perfloxacin (49.4%), Chloramphenicol (38.8%), and Ciprofloxacin (36.5%).

Figure 1: Prevalence of *E.coli* among pregnant women in selected hospitals within Kaduna metropolis.

Table 1: Microgen GnA identification of *E. coli* isolates obtained from urine sample collected.

Sample Code	Octal Code	% Probability	Identification
GHS1-10	6770	92.97%	<i>E. coli</i>
BDSH11-30	6561	90.18%	<i>E. coli</i>
YDM31-60	6760	97.11%	<i>E. coli</i>
GHK61-75	6560	90.21%	<i>E. coli</i>
GAGH76-85	6660	80.66%	<i>E. coli</i>

Table 3: Distribution of *E.coli* Infection in relation to socio demographic and risk factors

Factors	No Examined	No Positive (%)	χ^2	P-value
Age-group				
15-20	90	17(18.9)	7.331	0.119
21-25	85	21(24.7)		
26-30	113	32(28.3)		
31-35	60	11(18.3)		
36-40	52	4(11.5)		
Social Economic Status				
Student	93	19(20.4)	12.891	0.005*
Civil servant	89	13(14.6)		
Self employed	100	14(14.0)		
Unemployed	118	37(31.4)		
Parity				
First pregnancy	125	20(16.0)	36.868	0.0*
Second pregnancy	170	21(12.4)		
Third pregnancy and above	105	44(41.9)		
Trimester				
First	60	10(16.7)	0.961	0.618
Second	240	52(21.7)		
Third	100	23(23.0)		
Toilet type				
Water cistern	242	17(7.0)	74.084	0.0*
Pit latrine	158	68(43.0)		

Key: χ^2 = Chi-square, P= prevalence

Table 4: Distribution of *E coli* Infection in relation to symptoms

Symptoms	No Examined	No Positive (%)	χ^2	P-value	Odds ratio
Discharges					
Yes	152	35(23.0)	0.462	0.530	1.185
No	248	50(20.2)			
Abdominal pains					
Yes	210	44(20.9)	0.023	0.903	0.963
No	190	41(21.6)			
Fever					
Yes	50	16(32.0)	3.946	0.047*	1.916
No	350	69(19.7)			
Vomiting/Nausea					
Yes	154	38(24.7)	1.756	0.185	1.387
No	246	47(19.1)			
Back ache					
Yes	83	27(32.5)	7.963	0.005*	2.070
No	367	48(18.3)			
Itchy sensation					
Yes	72	26(36.1)	11.588	0.001*	2.577
No	318	49(15.4)			

Key: χ^2 = Chi-square, P= prevalence

Table 5: Antimicrobial susceptibility patterns of *E. coli* isolates from urine samples

Antibiotics/potency (µg)	Resistant (%)	Intermediate (%)	Susceptible (%)
Cotrimoxazole (30)	27(31.8)	10(11.8)	48(56.5)
Chloramphenicol (30)	33(38.8)	14(16.5)	38(44.7)
Sparfloxacin (10)	22(25.9)	20(23.5)	43(50.6)
Ciprofloxacin (10)	31(36.5)	15(17.6)	39(45.9)
Amoxycillin (30)	18(21.2)	7(8.2)	60(70.5)
Augmentin (30)	24(28.2)	16(18.8)	45(52.9)
Gentamycin (10)	20(23.5)	6(7.1)	62(72.9)
Perfloxacin (30)	42(49.4)	2(2.4)	41(48.2)
Tarivid (10)	21(24.7)	7(8.2)	47(55.3)
Streptomycin (30)	25(2.4)	0(0.0)	60(70.5)

DISCUSSION

Urinary tract infection (UTI) occurs as a result of microbial colonization of urine and the invasion of any structure of the urinary tract by microorganism such as bacteria, viruses, yeast and parasite (Okafor and Elenwo, 2008). This study has isolated *Escherichia coli* as the common bacterial agent infecting pregnant women within Kaduna metropolis.

The prevalence of *Escherichia coli* was found to be 21.3%. The high prevalence of *Escherichia coli* reported in this study may be attributed to the environmental condition where the subjects reside. This may also be attributed to lack of proper personal and environmental hygiene, genuine population susceptibility since this factors such as low social economic status, sexual intercourse, pregnancy among others are common among Nigerian men and women (Andriole, 1985; Akinyemi *et al.*, 1997; Kolawole *et al.*, 2009). *Escherichia coli* infection is mostly common with UTI. The value obtained is similar to 26% reported in Iran, (Rekebizadeh and KhodaVandi, 2017). However, it is higher than 16% reported in Ibadan (Okonko *et al.*, 2010) and 8.0% reported in Kaduna (Mohammed, 2015). The result is lower compared to 46.7% reported in Ilorin, Nigeria (Idris *et al.*, 2014) and 34.2% reported by Iroha *et al.*, (2017) in Ebonyi state, Nigeria.

Higher prevalence of infection was reported among pregnant women within the age of 26-30. The reason for the high prevalence within this age group is due to the fact that most pregnancy occurs averagely within this age group. Most of the pregnant women examined were unemployed (housewives) and they had the highest prevalence of *E. coli* infection. This may be due to nonchalant attitude towards basic and personal hygiene. However, our findings disagree with (Okonko *et al.*, 2010) where low incidence of the infection was reported among the housewives.

The findings of this study showed that 23% of the women who had UTI were in their 3rd pregnancy and above. This shows that high parity is one of the possible factors affecting the prevalence of urinary tract infection among pregnant women (Okafor and Elenwo, 2008).

In this study, women in their 2nd and 3rd trimester were found to have a higher prevalence of *Escherichia coli* infection; 21.7% and 23.0% respectively.

There was statistical significant associated between symptoms such as fever, backache, and pruritis and *E. coli* associated with UTI; $p=0.047$, 0.005 and 0.001 respectively. Most of the pregnant women had fever, backache and pruritis.

The antibiotic susceptibility pattern of *E. coli* isolated in this study showed that Gentamycin is an effective antibiotic having a susceptibility of 72.9%. This result is similar to that of Ayoade *et al.*, (2013) and Nteziyaremye *et al.*, (2020). Also, Amoxycillin and Streptomycin were considerable effective agreeing with the report of other studies (Teklu *et al.*, 2019; Tedesse *et al.*, 2014 and Andabati, 2010).

The resistance of *Escherichia coli* to most of the antibiotic in this study may reflect the fact that they are common antibiotic taken without proper prescription. Also, this might be an indication of the fact that these isolate have had previous exposure to these antibiotics.

The emergence of multi drug resistance (MDR) has been associated with wide spread use of antibiotics, inappropriate drug use and weak antibiotic monitoring. This lead to selection of antibiotic resistance mechanism in bacteria. The emergence of MDR is a serious problem. It compromises the activity of the broad spectrum antibiotics and is a major therapeutic challenge with great impact on patient outcomes (Jyoti *et al.*, 2014).

CONCLUSION

The prevalence of *Escherichia coli* in association with UTI among pregnant women in selected hospitals within Kaduna metropolis was 85 (21.3%). The *Escherichia coli* isolates

showed highest susceptibility to Gentamicin, Amoxycillin and Streptomycin. However, the isolates showed higher resistance to Perfloxacin, Chloramphenicol, and Ciprofloxacin.

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