

Arid Zone Journal of Basic and Applied Research

Faculty of Science, Borno State University Maiduguri, Nigeria

Journal homepage: https://www.azjournalbar.com



Research Article

Prevalence and Antibacterial Susceptibility of Isolates of Coliuria among Pregnant Women Attending Antenatal Clinic at State Specialist Hospital, Maiduguri

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ARTICLE INFO:

ABSTRACT

Keywords:

Coliuria, E. coli, Antenatal, Pregnant women, Antibacterial Urinary tract infection (UTI) is common among pregnant women and can be associated with negative outcome for both the mother and the foetus. In view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary to improve guideline for empirical antibacterial therapy. The aims of this study were to estimate prevalence of coliuria and determine the antibiogram of the isolates among pregnant women attending antenatal clinics at State Specialist Hospital, Maiduguri. This study involved pregnant women at any stage of gestation attending Antenatal Clinics at State Specialist Hospital, Maiduguri without signs of life-threatening conditions and focused on E. coli as a cause of UTI using urine samples. Plate streaking method was used in isolation of E. coli while disc diffusion method was used as technique for antibacterial susceptibility testing. The urine samples analysed showed a prevalence of coliuria of 39.1 % (59/151) among the subjects. The results of antibacterial sensitivity indicated that gentamicin (86.4%, 51/59) demonstrated significantly (p < 0.05) highest activity against the isolates while streptomycin (10.2%, 6/59) and cephalexin (10.2%, 6/59) had the least activity. The antibacterial resistance of individual isolates revealed that most of the isolates were multi-drug resistant strains and majority (98.3%, 58/59) of the isolates was resistant to at least three (3) antibacterial drugs. Isolate 11 (Ec11) was resistant to all the ten (10) antibacterial drugs tested while isolate 35 (Ec35) had the least resistance of 10% as it was only sensitive to gentamicin. These findings suggest that there is a high prevalence of coliuria among pregnant women attending antenatal clinics at SSH, Maiduguri. Only gentamicin is suitable antibacterial agent in the treatment of the condition and this could contribute to effective treatment and effective discharge of antenatal care practice in Maiduguri.

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INTRODUCTION

The proximity of the outlets of the urogenital and GIT systems promote exchange of bacterial contaminants of both systems, thus, E. coli sheds in faeces may contaminate the outlet especially urogenital in (Tazebew et al., 2012). This may ascend the urinary tract to cause urinary tract infection (UTI) especially among immunocompromised individuals including pregnant women (Gessese et al., 2017). In addition, some pathogenic strains of E. coli are recognized to cause UTI, these are called uropathogenic Е. colie.g. serotypes O18:K1:H7 (Johnson et al., 2001). Urinary tract infection due to E. coli does not always cause clinical signs but usually manifest as coliuria - presence of E. coli in urine. However, when it does they may include: strong persistent urge to urinate, urine appears cloudy, pelvic pain in women, burning sensation when urinating and passing frequent, small amount of urine (Bennett et al., 2015). UTI is common during pregnancy and can be associated with negative outcome for both the mother and the foetus such as preterm labour, preeclampsia, amnionitis and anaemia among others (Mazor-Dray et al., 2009). The incidence is higher among women who have had childhood infections as reported by Martinell et al. (1990). It has been observed that pregnant women have a propensity to develop recurrent UTI (Nowicki, 2002). Considering the risk factors associated with the infection, UTI screening should be considered during antenatal clinics by assessment of urine samples for presence of Treatment bacteria. of most infections (including UTI) is often started empirically without adequate knowledge antibacterial sensitivity of the causative agents to guide therapy. Thus, treatments are often treated with broad-spectrum antibacterial drugs such as ciprofloxacin, norfloxacin, metronidazole, erythromycin etc. In view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary

to improve guideline for empirical antibacterial therapy (Grude *et al.*, 2001).

The medical importance of E. coli cannot be over emphasised, especially) as uropathogenic organism among pregnant women in sub-Saharan Africa. For instance, Derese et al. (2016) reported prevalence of 14% bacteriuria among pregnant women, of which 34.6% was due to E. coli. Similarly, significant prevalence of UTI was reported from Sudan and Tanzania (Masinde et al., 2009; Hamdan et al., 2011) Ε. colicontributing significant proportion. In Nigeria, E. coli has been reported in urine samples of pregnant women in Benin, Ebonyi and Ile-ife (Akerele et al., 2001; Ade-ojo et al., 2013; Onuoha et al., 2014). In Maiduguri, Okon et al. (2012) reported presence of number of bacteria in urine samples of apparently healthy and unhealthy individuals. Most of these studies are domiciled in University of Maiduguri Teaching Hospital (UMTH) with little or no special attention given to pregnant women (despite their risk status) and E. coli. Thus, there is paucity of evidence-based data on coliuria among pregnant women attending other hospitals in Maiduguri. To this end, the study was designed to isolate E. coli and determine the antibiogram among pregnant women attending antenatal clinic at State Specialist Hospital (SSH), Maiduguri.

MATERIALS AND METHODS Study Area

The study was carried out at State Specialist Hospital (SSH), Maiduguri. SSH is located at Shehu Lamimu Way, Post Office Area, Maiduguri, Borno State, Nigeria. It is a 500bed capacity tertiary hospital with 15 wards and various clinical departments such as Medicine, Paediatric, General Outpatient, Laboratory Physiotherapy, Service Radiography Departments. It is a major referral center within and outside the state; it also serves as a primary health center for some individuals. The antenatal clinics are run three times (Mondays, Wednesdays and Thursdays) in a week with over one hundred (100)

pregnant women seen on each day. The laboratory analysis of the urine sample was done at Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri.

Study Population

One hundred and fifty-one (151) consecutive pregnant women attending antenatal clinics at SSH, Maiduguri were enrolled between March and April, 2017 following an informed consent. The study was based on the following criteria: pregnancy of any stage of gestation, attendance of antenatal clinic at SSH, Maiduguri, ability to obtain informed consent and apparently healthy individuals or presence of mild-moderate symptoms of ill-health. The subjects were excluded based on the following criteria: failure to secure informed consent, evidence of complicated pregnancy requiring urgent attention and presence of severe illhealth. A standard case record form was used obtain demographic and medical information of each of the enrolled subjects.

Sample Collection and Preservation

Mid-stream early morning urine sample was collected from all enrolled subjects as described by Cheesbrough (2006). The sample was collected by the subjects following detailed description on how to collect uncontaminated urine sample by researcher. Briefly, the subjects were asked to void the first portion of the urine into the toilet and collect the mid-stream portion into a labelled universal sterile container, and the remaining urine was voided into the toilet. After collection, the urine samples were preserved in the fridge at a temperature of 4°C till the end of sampling for each day which was not longer than 6 hours. The urine samples were transported in cold pack to the laboratory where the analysis commenced immediately on arrival. The transport between SSH and the laboratory took average of 30 minutes.

Urinalysis

Urinalysis was done as described Cheesbrough (2006) and Boye et al. (2012) using urine test strip (Combi-9[®]). Firstly, physical examination was done to determine the colour of each sample. Then, urine test strip was dipped into each sample for one (1) minute and allowed to stay for 3 minutes after removal before the readings were taken. The urine clarity, pH, nitrite and protein contents were determined by comparing the observed colour changes on the strip with reference colour chart provided on the test strip container.

ISSN: 2811-2881

Culture and Isolation of E. coli

E. coli was isolated by culture morphology and biochemical test as described by Cheesbrough (2006) with slight modification to meet the available resources. Briefly, the urine sample was brought to room temperature by placing the container on laboratory bench for about one (1) hour. Then, a loop-full of each urine sample was aseptically streaked MacConkey agar and aerobically incubated at 37°C for 24 hours. The incubation of plates without growth after 24 hours was extended to 48 hours before declaring negative. E. coli was tentatively suspected based on the morphology of the colonies (non-mucoid and lactose fermenting) where growth was observed. The mucoid lactose fermenters were recovered and subjected to indole and motility tests for identification of *E. coli* from isolates.

Indole and Motility Test

Indole and motility tests were conducted as described by Cheesbrough (2006)MacFaddin (2000) to differentiate E. coli from other non-mucoid fermenters. A small portion of the non-mucoid lactose fermenters among the bacterial colonies was inoculated in a Bijou's bottle containing 3 ml of peptone water. This was incubated at 37°C for 24 hours. For motility test, a drop of the incubated peptone water with the bacterial inoculum was placed on a clean, grease-free microscope slide and covered with a cover slip. Motility was observed using light microscope under

objective lens ×10 and ×40. Observation of motility indicated *E. coli* (MacFaddin, 2000; Cheesbrough, 2006).

For indole test, 0.5 ml of Kovac's reagent was added to the incubated peptone water containing the inoculum and was shaked. Presence of red layer at the surface (ring formation) indicated positive result for *E. coli* (MacFaddin, 2000; Cheesbrough, 2006).

Antibacterial Sensitivity Test

The antibacterial sensitivity of the E. coli isolates was determined by Kirby-Bauer disc diffusion method as described by Cheesbrough (2006) with slight modification (nutrient agar was used in place of Muller-Hinton agar). The inoculum of each isolate was prepared in peptone water and was aseptically inoculated into the nutrient agar by spread plate method. The antibacterial discs were gently and aseptically placed on the media and pressed down slowly with the aid of a sterile forceps to ensure adequate contact with the media. The plate was then incubated at 37°C for 24 hours and the zones of inhibition measured using divider and scale rule. The zones of inhibition were interpreted as sensitive, intermediate and resistant as described by Cheesbrough (2006).

Statistical Analysis

The data were analyzed using the statistical software; Statistical Package for Social Sciences (SPSS, 2007). The results were presented in texts, tables and figures where necessary and expressed as percentages, mean, standard deviation and range. Proportions were compared using Chi-square, while the mean values were compared using analysis of variance. Significance was inferred at $p \le 0.05$.

RESULTS

Profile of the Subjects

Table 1 presents the demographic profile of the 151 subjects who participated in the present study. The mean of the subjects' age was $26.3 \pm 6.0 (16 - 47)$ years with age group 20 - 29 years accounted for highest proportion of (60.9%, 92/151) [p < 0.05]. The mean of gestational age was $7.7 \pm 1.3 (4 - 10)$ months, significant majority (77.5%, 117/151) [p <

0.05] of the subjects were at third trimester. On the average, the subjects have attended Antenatal Clinics three (3) times at the time of enrolment into the study.

Symptoms Presented by Subjects at Enrolment

The proportion of enrolled subjects with presenting symptoms (46.4%; 68/151) could be statistically insignificant when compared with subjects without presenting symptoms (53.6%; 83/151) [p > 0.05]. The proportion of various symptoms presented by the 68 subjects with symptoms at enrolment is presented in Table 2. Abdominal discomfort has significantly (p < 0.05) highest proportion of 30.9% (21/68). Only one (1) subject had painful urination, a possible indication of clinical urinary tract infection.

Characteristics of the Urine Samples

Table 3 presents selected physical and chemical characteristics of the urine samples. Significantly (p < 0.05) lowest proportions of the urine samples was colourless (1.3%; 2/151) or had red colour (0.7%; 1/151) while significantly (p < 0.05) highest proportion of the samples had clear appearance (66.2%; 100/151). In addition, majority of the samples had no proteinuria (63.6%, 96/151, p < 0.05) and no nitrite present (81.4%, 123/151, p < 0.05). The proportion of the samples with neutral pH (59.6%, 90/151) was significantly (p < 0.05) highest than samples with acidic (35.1%, 53/151) and alkaline (5.3%, 8/151) pH.

Coliuria among the Subjects

Of all the 151 urine samples analysed, *E. coli* was isolated from 59 samples giving a prevalence of 39.1% (59/151) of coliuria among the subjects. The factors associated with coliuria among the subjects were evaluated and presented in Table 4. Of all the parameters analyzed, gestational age, parity and presence of symptoms were associated with coliuria among the subjects, that is, subjects at second trimester of gestation (p < 0.05), those with at least parity of three (p <

0.05) and symptomatic are most likely to have coliuria.

Antibiogram of the E. coli Isolates

The antibacterial susceptibility of the 59 E. coli clinical isolates was determined using ten (10) antibacterial drugs. Figure 1 shows the antibiogram of the 59 E. coli isolates recovered from the urine samples. The overall mean of antibacterial sensitivity (31.9 \pm 7.9) was statistically insignificant compared with antibacterial resistance (39.0 \pm 10.7) [p > 0.05]. The results also showed that gentamicin (86.4%, 51/59) demonstrated significantly (p < 0.05) highest activity against the isolates while streptomycin (10.2%, 6/59) and cephalexin (10.2%, 6/59) had the least activity. Further analysis of the antibacterial resistance of individual isolates revealed that most of the isolates were multi-drug resistant strains. Majority (98.3%, 58/59) of the isolates were resistant to at least three (3) antibacterial drugs. Isolate 11 (Ec11) was resistant to all the ten (10) antibacterial drugs tested while isolate 35 (Ec35) had the least resistance of 10% as it was only sensitive to gentamicin.

DISCUSSION

E. coli remains one of the most important bacteria in the public health. Many strains of E. coli have been described as non-pathogenic normal flora of gastrointestinal tract of humans and animals (WHO, 2017). Yet, other strains have been reported as causative agents of various infections especially among children, immunocompromised individuals pregnant women (Kaper et al., 2004). One of such infections is urinary tract infections (UTI) studies where previous have largely acknowledged the contributions of E. coli to morbidity and mortality of UTI (Johnson et al., 2001). While UTI in non-pregnant women is generally benign, pregnant women with bacteriuria have an increased susceptibility to serious conditions such as pyelonephritis (Dafnis and Sabatini, 1992). The present study examined coliuria among pregnant women attending antenatal clinics at State Specialist Hospital, Maiduguri and determined the antibiogram of the *E. coli* isolates recovered from the urine samples.

In the present study, approximately half of the pregnant women studied were asymptomatic while the remaining half presented nonspecific symptoms. This is in accordance with previous studies which reported that UTI is mostly asymptomatic especially among pregnant women (Smaill and Schnarr, 2008; Boye et al., 2012) but may become symptomatic if untreated (Kalantar et al., 2008). The fact that most of the urine samples in the present study were either clear or turbid does not indicate the presence or absence of UTI. Turbid urine is often a common feature in the morning and it may be due to lowering of the water level in the body. However, when it is accompanied by other symptoms like burning sensation when passing urine, frequent urge to urinate etc., then UTI might be suspected (Bennett et al., 2015). Meanwhile, detection of protein and nitrite in certain proportions of the samples may be a pointer towards bacteriuria. Gram-negative organisms like E. coli are able to reduce nitrate present in urine to nitrite, hence could account for the nitrite present. This finding agrees with previous reports that the presence of protein and nitrite in urine is an indication of bacteria invasion (Cheesbrough, 2006; Boye et al., 2012).

The prevalence of coliuria among the pregnant women observed in the present study is relatively high; while the prevalence is comparable to some of the previous studies conducted within and outside Nigeria; it varies greatly from few others. For instance, the present value (39.1%) is significantly higher than 11.5% reported by Okon et al. (2012) from another Tertiary Hospitals in Maiduguri. This discordance could be attributed to the fact that Okon et al. (2012) quantified the colonies and exempted those with values below 10⁵ CFU/ml. In contrast, the present value is similar to 30.7% reported in Lafia and 47.5% in Ilorin, North-central Nigeria (Abdul and Onile, 2001), 38% in Benin city, South-south

Nigeria (Onuh et al., 2006) and 42.2% in Ileife, Southwest Nigeria (Ezechi et al., 2003). Similarly, this study is in accordance with previous findings from outside Nigeria, with 27.5% and 38% in Ghana and Pakistan, respectively (Aziz et al., 2006; Boye et al., 2012). In addition, Derese et al. (2016) reported prevalence of 14% bacteriuria among pregnant women, of which 34.6% was due to E. coli. The fact that coliuria was associated with second trimester, multi-parity presence of symptoms was not surprising. Previously, Boye et al. (2012) and Onuh et al. (2006) have attributed second trimester with highest prevalence of bacteriuria and Okon et al. (2012) reported highest prevalence among multiparous pregnant women. This could be attributed to the anatomical and physiological changes experienced during late stages of gestation such as expansion of uterus, hormonal and homeostatic changes which favour bacterial invasion (Boye et al., 2012). Furthermore, the fact that most of the E. coli isolates were of muti-drug resistant strains raised major concern of public health interest. Thus, preventive measures must be employed to halt the spread of the isolates. This finding is in accordance with previous studies that have shown that E. coli strains circulating in most hospital and community settings are resistant to number of antibacterial drugs (Boye et al., 2012; Okon et al., 2012; Akobi et al., 2014). The level of resistance could be attributed to irrational antibacterial drugs. E. coli is a Gram-negative bacteria, thus, it is not uncommon that gentamicin exhibited sufficient antibacterial activity against the isolates. In addition, this could be an indication of reduced gentamicin pressure.

CONCLUSION

In conclusion, the prevalence of coliuria among pregnant women attending antenatal clinics at SSH, Maiduguri, is relatively high. Although this may not be an indication of clinical infection since the *E. coli* colonies were not quantified. The coliuria was

associated with gestational age at second trimester, multi-parity and presence of symptoms. In addition, the *E. coli* isolates recovered from the urine samples of the subjects were mostly multi-drug resistant strains. Only gentamicin demonstrated appreciable level of activity against the isolates. These findings could contribute to effective discharge of antenatal care practice in Maiduguri.

AUTHORS' CONTRIBUTIONS

BST and AAO conceptualized the study and participated in drafting of the manuscript, BST DHH, AAO and PLM did statistical analysis and assisted in designing the study as well as drafting of the manuscript and BST and DHH implemented the study (acquisition of data) and interpreted the data. SOA revised the draft critically for intellectual content and gave final approval of the version to be submitted.

FUNDING

This study was not covered by any grant but was self-financed.

ETHICS APPROVAL

The ethical approval for the study was obtained from the Research Committee of SSH, Maiduguri. The study was conducted according to best clinical and laboratory practices. Informed consent was obtained from each subject prior to enrolment

CONFLICT OF INTEREST

No conflict of interest.

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Table 1: Demographic profile of the 151 enrolled subjects

Parameter	Value	
Age (years)	$26.3 \pm 6.0 (16.0 - 47.0)$	
Weight (kg)	$67.7 \pm 11.7 \ (35.0 - 105.0)$	
Height (cm)	$160.2 \pm 6.4 (145.0 - 175.0)$	
Gestational age (months)	$7.7 \pm 1.3 \ (4.0 - 10.0)$	
Parity	$3.4 \pm 2.2 \ (1.0 - 11.0)$	
Age of ANC start (months)	$5.8 \pm 1.2 (3.0 - 8.0)$	
Number of ANC visit	$2.9 \pm 1.1 \; (1.0 - 7.0)$	

Values are mean \pm standard deviation (range)

ANC Antenatal Clinic

Table 2: Symptoms presented by the subjects at enrolment

Symptom	Frequency	*Percentage (%)
Abdominal discomfort	21	30.9
Back pain	11	16.2
Chest pain	2	2.9
Dizziness	7	10.3

Fever	4	5.9	
Headache	6	8.8	
Heart burn	2	2.9	
Infection	1	1.5	
Leg pain	8	11.8	
Nausea and vomiting	2	2.9	
Painful urination	1	1.5	
Palpitation	2	2.9	
Weakness	6	8.8	

ISSN: 2811-2881

^{*}Expressed over the total number of subjects with symptoms (68 subjects)

Table 3: Characteristics of the urine samples

Parameter	Frequency	Percentage (%)
Colour		
Amber	32	21.2
Colourless	2	1.3*
Light amber	34	22.5
Pale yellow	52	34.4
Red	1	0.7*
Yellow	30	19.9
Clarity		
Clear	100	66.2*
Cloudy	6	4.0
Slightly cloudy	10	6.6
Turbid	35	23.2
Proteinuria		
Yes	55	36.4
No	96	63.6*
Nitrite		
Yes	28	18.5
No	123	81.4*
pH	-	
1-6	53	35.1
7	90	59.6*
8-14	8	5.3

^{*}p < 0.05 when compared with others

Table 4: Factors associated with coliuria among the subjects (n=151)

	Number Numb	Number with	Odd Ratio	S
Parameter	Enrolled	Coliuria (%)	(95% CI)	p value
Age (years)				
< 20	12	4 (33.3)	1.0	> 0.05
20-29	92	33 (35.9)	1.1 (0.9-1.3)	
30-39	45	21 (46.7)	1.4 (1.1-1.7)	
≥ 40	2	1 (50)	1.5 (1.3-1.7	
Gestational age				
2 nd Trimester	34	18 (52.9)	1.0	< 0.05
3 rd Trimester	117	41 (35.0)	0.7 (0.5-0.9)	
pН				
1-6	53	23 (43.3)	1.0	> 0.05
7	90	33 (36.7)	0.9 (0.5-1.3)	
8-14	8	3 (37.5)	0.9 (0.5-1.3)	
Clarity				
Clear	100	42 (42.0)	1.0	> 0.05
Not-clear	51	17 (33.3)	0.8 (0.6-1.0)	
Colour				
Amber/pale amber	66	20 (43.5)	1.0	> 0.05
Colourless	82	38 (46.3)	1.1 (0.8-1.4)	
Red	2	0 (0.0)	-	
Yellow/pale yellow	1	1 (100.0)	-	
Parity				
≥ 2	63	19 (30.2)	1.0	< 0.05
>2	88	40 (45.5)	1.5 (1.2-1.8)	
Symptoms				

Yes	58	28 (48.3)	1.0	< 0.05
No	93	31 (33.3)	0.7 (0.5-0.9)	

ISSN: 2811-2881

CI Confidence Interval

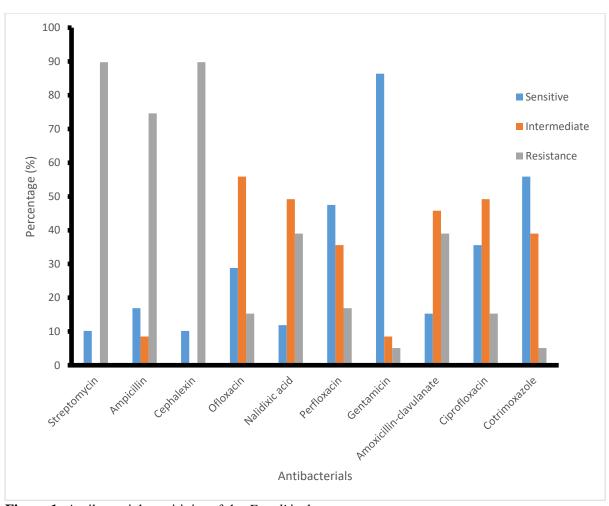


Figure 1: Antibacterial sensitivity of the *E. coli* isolates