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OCCURRENCE AND ANTIBIOGRAM PATTERN OF *Escherichia coli* ISOLATED FROM THE URINARY TRACT OF PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN ILORIN METROPOLIS

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ABSTRACT

Urinary Tract Infection (UTI) is a serious health problem affecting millions of people globally. In females, it is more common during pregnancy and is characterized by increased morbidity, maternal and perinatal complications. This alarming rate of UTI related complications and antibiotics resistance necessitated this study among pregnant women attending ante – natal clinics within Ilorin metropolis of Kwara State, Nigeria. A cross-sectional study involving 420 pregnant women attending ante – natal clinics was conducted from February, 2018 to April, 2019. Midstream urine samples were collected from enrolled subjects and cultured using quantitative urine culture method. Isolates were identified using standard bacteriological method, further confirmed using the Microgen Identification system and susceptibility testing was performed using the Disc Diffusion method. A total of 188 (44.67%) of the 420 urine samples had significant bacteriuria (colony-forming unit ≥10^5/ml), and 56 (26.05 %) of the isolates were characterized as *Escherichia coli*. The *E. coli* isolates exhibited high resistant to ampicillin, tetracycline, amoxicillin clavulanic acid, cefuroxime sodium at 88.68 %, 73.59%, 69.81% and 66.04% respectively. Of these isolates, 38 (71.70%) were resistant to three or more antibiotics and regarded as multidrug resistant. The most occurring resistant phenotype was ampicillin 8 (15.09%), followed by its combination with amoxycillin clavulanate, cefuroxime sodium 7 (13.21%) as well as tetracycline and sulphamethoxazole trimethoprim 6 (11.32%). All *E. coli* isolates were sensitive to imipenem and nitrofurantoin. This study indicates high prevalence of multidrug resistant *E. coli* in pregnancy associated UTIs with potential health consequences.

KEYWORDS: **Antibiogram pattern; *Escherichia coli*; Pregnant women; Urinary tract infections (UTIs).**

INTRODUCTION

Urinary Tract Infections (UTIs) are commonly encountered in clinical practice affecting over 150 million persons of all age groups globally [1]. Most frequently, UTIs occur in adult women with about 48% showing symptoms suggestive of UTI or bacterial cystitis during their lifetime as compared to 1 in 20 men [2]. This higher incidence in women is due to the shorter urethra, closer proximity of the anus to the vaginal vestibule and urethra that allows easy transfer of bacteria within the tract [3]. Other factors include state of immunity, dysfunctional pelvic floor, altered vaginal biota, [4] family history of

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UTI, menopause, diabetes, pregnancy [5] and sexual intercourse [6].
UTIs are the most common bacterial infections during pregnancy [7] that may be manifested as asymptomatic bacteriuria (ASB), symptomatic bacteriuria (SB) in form of pyelonephritis, cystitis, sepsis and urethritis [8]. This is due to the general changes in the body during pregnancy which increases the risk of infections [9]. Physical, immunological, hormonal and structural changes during pregnancy which increase the onset of UTIs and complications when untreated [9, 10]. About 5 – 10% of ASB may progress to symptomatic bacteriuria which could lead to acute pyelonephritis in 20-50% of pregnant women contributing significantly to maternal and perinatal outcomes [10]. *Enterobacteriaceae* especially the *Escherichia coli* are mainly incriminated [6], in about 80% - 90% of UTIs in pregnancy, 85% of community acquired UTIs, 50% of nosocomial UTIs and more than 80% of uncomplicated pyelonephritis [10]. Antimicrobial resistance among *E. coli* associated with UTIs is currently a major cause of increasing antimicrobial resistance [11, 12] thus prolonging disease morbidity, cost of treatment, hospitalization and in severe cases resulting to death [1].
Understanding the occurrence of *E. coli* in UTI and their antibiogram patterns is very important for optimal empirical therapy especially with changing prevalence, etiological agents and antimicrobial patterns. Previous studies have reported the prevalence and antimicrobial resistance patterns of uropathogens isolated from pregnant women within Ilorin [13 -16]. This study seeks to determine the occurrence and antibiogram patterns of characterized *E. coli* isolates associated with UTI in pregnant women within Ilorin with the aim to design an effective empirical treatment intervention for this vulnerable group.

MATERIALS AND METHODS

Study area

The study was conducted within Ilorin Metropolis. Ilorin Metropolis consists of three Local Government Areas namely; Ilorin West, Ilorin East and Ilorin South as indicated (Figure 1) with a land size of about 150sq km and a population of 777,667 inhabitants [17]. The metropolis harbors three categories of hospitals namely; tertiary, secondary and primary (public or private) health centers [18].

Samples and sampling techniques

Purposive and convenient sampling techniques were used for the selection of health facilities with ante – natal clinics and pregnant women respectively.

Study population

A cross-sectional study of pregnant women attending selected antenatal clinics in selected health centers within Ilorin metropolis, Kwara State was conducted. From the three categories of hospitals (Tertiary, Secondary and Primary), seven (7) centers with antenatal clinics were selected for the study. Only pregnant women who were not on antibiotics within the time of recruitment and those who were willing were conveniently recruited for the study.

Ethical / consent considerations

Ethical clearance was obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital and State Ministry of Health (UITH/CAS/ 189/19^{B}/789 and MOH/ KS/EU/777/206). Prior to sample collection, permission was obtained from the Head, Department of Obstetrics and Gynecology housing the Ante – natal units. In addition, oral consent was obtained from the pregnant women that met the study criteria.

Sample size and sample collection

Sample size was determined using the statistical method [19] with the formula the $N=Z^2pq/L^2$ and 40% prevalence UTI rate as reported in Ilorin [14]. A total of 420 urine samples were collected from 7 (seven) selected ante – natal clinics between February, 2018 to April, 2019.
Mid – stream urine samples were collected in wide – mouthed sterile universal containers [20]. Description on how to collect samples and the need for prompt delivery was emphasized to participants. On delivery, urine samples were carefully labeled and transported in cold chain to the Pharmaceutical Microbiology and Biotechnology Laboratory, University of Ilorin for immediate analysis.

Isolation and identification of isolates

This was performed using quantitative urine culture method [20, 21]. Using calibrated wire loop one (1) µL (0.001mL) clean-catch midstream urine samples were inoculated into Cystein Lactose Electrolyte Deficiency (CLED), Eosin Methylene Blue agar (EMB) and MacConkey Agar (MCA) (Oxoid, Basingstoke, UK). All plates were incubated aerobically at 37°C for up 24hours. CLED plates yielding bacterial growth of $\geq 10^5$ CFU/mL of urine was considered as significant for bacteriuria according to the Infectious Diseases Society of America (IDSA) guidelines reported by Nicolle [22].
Colonies on MCA and EMB were presumptively identified based on colonial morphology, Gram staining and oxidase test in accordance to the

standard bacteriological protocol described by Cheesbrough [21]. Further identification of the oxidase negative gram bacteria was done using Microgen® rapid identification kit as specified by the manufacturer and confirmed on the Microgen Identification software [23].

Antibiotics susceptibility testing

Antibiotic susceptibility patterns of the bacterial isolates were evaluated using modified Kirby-Bauer discs diffusion method [24]. The antibiotic discs (Oxoid, Basingstoke, UK) of sulphamethoxazole Trimethoprim (cotrimoxazole) (25 µg), gentamicin (10 µg), ciprofloxacin (30 µg), amoxicillin (10 µg), imipenem (30 µg), nitrofurantoin (300 µg), amoxycillin clavulanate (30 µg), cephalexin (30 µg), cefuroxime sodium (30 µg) and tetracycline (30 µg) were used. Standardized freshly sub - cultured isolates of *E. coli* were inoculated aseptically onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) and the antibiotic discs were placed at equidistant. The MHA plates were then incubated at 37◦C for 24 hours. After 24 h incubation, the inhibition zones were measured in millimeter and interpreted based the recommendations of the Clinical Laboratory Standards Institute [25].

Determination of multiple antibiotic resistance (MAR) index

MAR Index was determined in accordance with the method described by Paul and others [26], using the formula MAR = x/y,

Where;

x = the number of antibiotics to which the bacterial isolate is resistant.

y = the total number of antibiotics to which the isolate has been evaluated for susceptibility.

Determination of resistance phenotypes

Resistance pattern was determined using the method described by Tsaku and colleagues [27]. This involved the determination and combination of the different antibiotic resistant patterns yielding different resistance phenotypes.

Statistical analysis

Data obtained were analyzed using simple descriptive statistics such as frequency, percentages, bar chart and tabular presentations.

RESULTS

The results showed that 52.0%, 35.5% and 54.7% of the urine samples collected from tertiary, secondary and primary health facilities respectively were positive for bacteriuria (Table 1). Significant bacteriuria was evident in 188 (44.67%) and *E. coli* isolates accounted for 26.05 % of the uropathogens. The antibiogram patterns of these *E. coli* isolates indicated highest resistances to amplicillin (88.68%), tetracycline (73.59%) and amoxicillin clavulanic acid (69.81%). Uropathogenic *E. coli* isolates were sensitive to imipenem and nitroforantoin at 100% (Figure 2). Table 2 shows that 71.70% of *E. coli* were resistant to 3 or more antibiotics with MAR index ranging from 0.1 – 0.9.

The highest MAR index was 0.4 (24.53%), least was 0.8 (1.89%) and none had an index of 1.0 that is resistant to all antibiotics used. Furthermore, twenty – three (23) different resistance phenotypes were observed in this study. The most prevalent phenotypes were AMP (15.09%), AMP, CXM and AMC (11.32%), AMP, TE and SXT (11.32%) and AMP and TE (9.43%) (Table 3).

DISCUSSION

In this study, the overall prevalence of UTI amongst pregnant women was 44.76 % similar to the 46.5 % reported in Abakiliki [28] but lower than the prevalence rates of 20 %, 21.0 % and 28.0 % that were reported in Mekkah [29], Benin [30] and Kaduna [31]. However, in Hodeida city of Yemen, Aba – Abia and Karu – Nassarawa State higher UTI prevalence rates of 54.5 %, 61.5 % and 62.67 % respectively were reported [32 – 34]. These shows that UTI prevalence rate varies globally and might be due to continuously changing associated factors such as geographical conditions, trends in antimicrobial resistance, time of study, characteristics of study population, sample size, cultural and socio – economic influence.

Previous studies in Ilorin reported UTI prevalence rates of 35.5 % in 2001 [13] and 27.90 % in 2019 [16] which are lower than the prevalence in this study. This increased prevalence rates over the years suggests an alarming health threat among pregnant women in Ilorin. The decrease prevalence from 2001 to 2019 may be ascribed to changes in study population characteristics, sample size and time of studies while the sudden increase from 27.90 % in 2019 to 44.76 % in this study most probably indicates a breakdown in personal and environmental hygiene practices.

The prevalence of uropathogenic *E. coli* (26.06%) observed in this study is similar to 26.1% reported in Southern Ethiopia [20]. This was lower than the 31.6% reported in 2001 by Abdul and Onile [13] and higher than the 2.9% by Olufadi – Ahmed *et al.* in 2019 [16] in the same study location. This changing

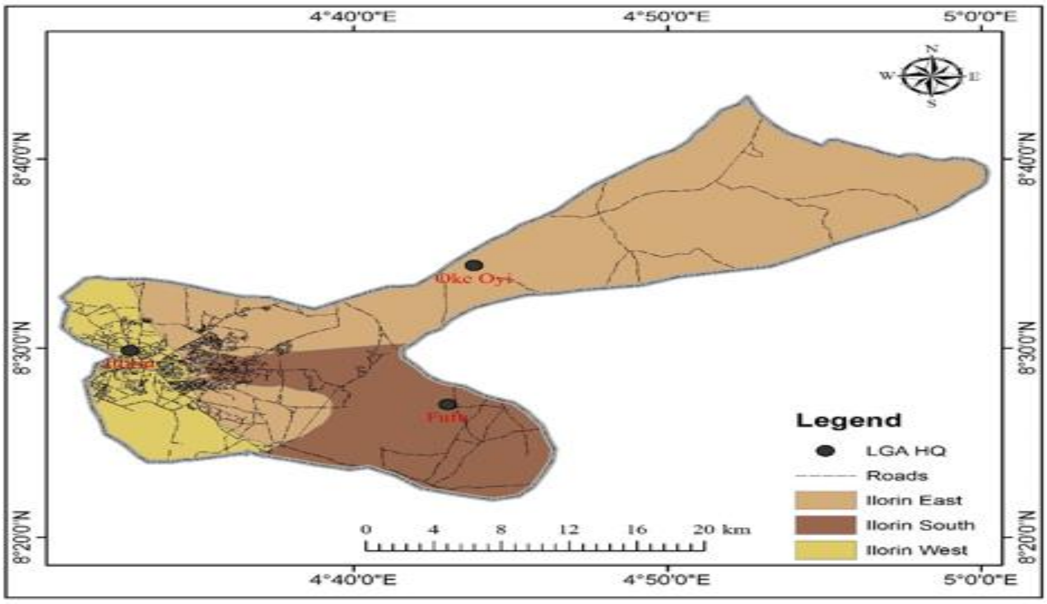

Figure 1: Map of Ilorin metropolis showing the different Local Government Areas (Map generated from Map Library, 2019 and coordinates obtained using Garmin ETrex 10).

Table 1: Distribution of samples collected from different heath facilities

Category of health Facility	No. of used health centers	No. of processed samples	No. of positive samples	%
Tertiary	1	100	52	52
Secondary	2	203	72	35.46
Primary	4	117	64	54.7
Total	**7**	**420**	**188**	**44.67**

Table 2: Multiple Antibiotic Resistance (MAR) Index of *E. coli* isolates

MARI	Number of Isolates (%)
0	4 (7.55)
0.1	11 (20.76)
0.2	0 (0.00)
0.3	9 (16.98)
0.4	13 (24.53)
0.5	10 (18.87)
0.6	2 (3.77)
0.7	0 (0.00)
0.8	1 (1.89)
0.9	3 (5.66)
1.0	0 (0.00)

n = 53

MAR Index of ≥ 0.3 = 71.70% of *E. coli* Isolates.

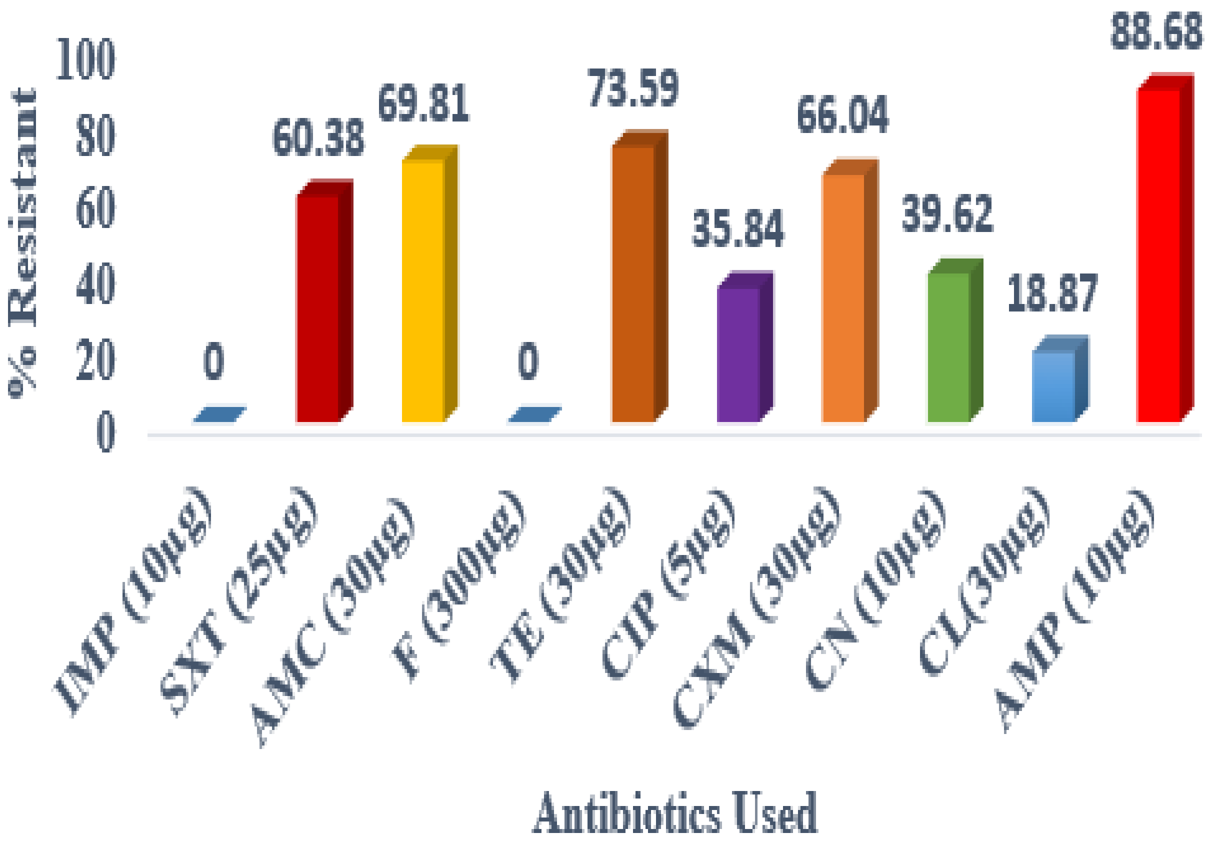

Figure 2: Antimicrobial resistance profile of *E. coli* isolated from pregnant women with UTI.

Keys:

IMP – Imipenem
SXT - Sulphamethoxazole trimethoprim
AMC - Amoxicillin clavulanic acid
F – Nirofurantoin
TE – Tetracycline
CIP – Ciprofloxacin
CXM - Cefuroxime sodium
CN – Gentamicin
CL – Cephalexin
AMP – Ampicillin.

Table 3: Resistance pattern of *E. coli* isolates from pregnant women with UTI

S/no.	Resistance pattern	Frequency	%
1	AMP	8	15.09
2	AMP - AMC – CXM	7	13.21
3	AMP - TE – SXT	6	11.32
4	AMP - TE	5	9.43
5	AMP - AMC - TE – SXT	4	7.55
6	AMP – AMC – CXM – TE – SXT – CN – CL – CIP	3	5.66
7	AMP – CN – CL	2	3.77
8	AMP – CXM	2	3.77
9	AMP – AMC – CXM – TE – SXT – CL – CIP	2	3.77
10	AMC	1	1.89
11	CN	1	1.89
12	AMP - AMC	1	1.89
13	AMP – CL	1	1.89
14	AMP – TE – CL	1	1.89
15	AMP – AMC – SXT	1	1.89
16	AMP – TE – SXT – CL	1	1.89
17	AMP – CXM – TE – CL	1	1.89
18	AMP – TE – SXT – CN	1	1.89
19	AMP – AMC – CXM – TE – SXT	1	1.89
20	AMP – TE – SXT – CL – CIP	1	1.89
21	AMP – AMC – TE – SXT – CL	1	1.89
22	AMP – AMC –TE – SXT – CN	1	1.89
23	AMP – AMC – CXM – TE – SXT – CIP	1	1.89
	TOTAL	**53**	**100**

Keys:

- Sulphamethoxazole Trimethoprim : SXT
- Tetracycline: TE
- Amoxycillin clavulanic acid : AMC
- Cephalexin: CL
- Gentamicin: CN
- Ampicillin: AMP
- Cefuroxime sodium: CXM
- Ciprofloxacin: CIP

prevalence of *E .coli* associated UTIs over the years might be due to unfavorable hygienic conditions, fecal contamination of foods and water within the study area. *E. coli* are especially of global health significance due to their supple nature to acquire genetic materials via vertical or horizontal gene transfer leading to the emergence of virulence strains of *E. coli* [35].

The uropathogenic *E. coli* isolates were resistant to ampicillin (88.68%). This result is similar with the report of 85% in Kano – Nigeria [36]. Lower rates of 39% and 53% were documented in India and Tanzania respectively [37, 38]. Although, several findings reported 100% resistance to ampicillin [39 - 41]. Other antimicrobial resistances recorded in this study is suggestive of the high rate of antibiotics misuse probably due to their cheap, readily availability or ease of administration. However, high resistance to penicillin and cephalosporin may be due to beta-lactamases production among *E. coli* [42] possibly confounding previous treatments with the resultant prevalence of infections in this study.

Furthermore, 71.70% of uropathogenic *E. coli* isolates in this study had MAR Index of ≥ 0.3. This could indicate high level of environmental contamination with antibiotics and bacteria that harbors resistant genes thereby increasing risk and complication of infections. Previous findings in Columbia stated a MAR Index of 70% from coliforms isolated from sewage outlet [43]. However, in Ekiti – Nigeria and Accra – Ghana, lower MAR Indices of 40% and 63% were reported among *E. coli* isolated from clinical and drinking water sources respectively [44 - 45]. Higher indices of 92.50% and 97.06% were reported among *E. coli* isolated from clinical and environmental samples from India and Nigeria [11, 27] suggesting varying antimicrobial resistances in different geographical locations which pose serious public health implications.

High antibiotics resistance phenotypes occurrence in this study disagrees with the report of Yu *et al.* [46] but agrees with the findings of Tsaku and others [27]. But a higher resistance phenotype amongst uropathogenic *E. coli* was reported in India [11]. This indicates the possibility of a wide diversity of *E. coli* circulation globally and within the Ilorin, posing serious health challenges and complexity for successful treatment of *E. coli* associated UTI thereby increasing disease, economic and social burden among this vulnerable group.

CONCLUSION

This study reports the prevalence rate of 44.76% of UTI among pregnant women in Ilorin. *E. coli* in this study environment are multiple drug resistant to frequently used antibiotics in the treatment of UTI posing challenge to successful outcome. However, imipenem and nitrofurantoin show promising treatment outcome for *E. coli* isolates from pregnancy associated UTIs.

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