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Prevalence of Multi Drug Resistance *Escherichia coli* Bacteriuria among Antenatal Women in a Tertiary Hospital in Enugu, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary Tract Infection (UTI) due to *Escherichia coli* (*E. coli*) is a common health problem among women. Pregnant women are at increased risk due to the physiological changes associated with pregnancy. The present study was designed to determine the prevalence of *E. coli* UTI and its antibiotic susceptibility pattern among pregnant women at the antenatal clinic of the Enugu State University of Science and Technology Teaching Hospital, Enugu, Nigeria.
Materials and Methods: A cross sectional study was carried out on a total of 200 pregnant women with and without symptoms of UTI. Mid-stream urine samples were collected and inoculated into Cysteine Lactose Electrolyte Deficient (CLED) and MacConkay. Colony counts > 102 cfu/ml of urine were regarded as significant bacteriuria. Pure isolates of bacterial pathogen were

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characterized by colony morphology, gram-stain and standard biochemical procedures. The method of agar disc diffusion susceptibility testing was used to determine the antibiotic resistance and sensitivity of the isolates.

Results: The overall prevalence of UTI was 15.4% while 48.5% were symptomatic cases and 5.0% asymptomatic cases. E,Coli was the most frequently isolated organism with a prevalence of 53.4% (111 samples) while other pathogens accounted for 46.6% (89 samples). *E. coli* showed the highest resistance to the antibiotic Ampicillin 100%, 78% to Tetracycline and 70% to Rocephine but a low resistance of 16.7% against Nitrofurantoin and Ceftriazone respectively.

Conclusion: UTI due to *E. coli* in pregnant women in Enugu is relatively high and shows high resistance to routine antibiotics. It is therefore, strongly recommended to undertake a routine culture as part of the antenatal care for pregnant women.

Keywords: UTI; E. coli; pregnancy; Enugu.

1. INTRODUCTION

"Urinary Tract Infection (UTI) is the invasion and multiplication of microorganisms that causes inflammation in any part of the urinary system" [1-3]. It affects individuals in all age groups and sexes and may be symptomatic or asymptomatic with females accounting for 87.5% of cases compared to males [4,5]. "Pregnant women are at increased risk due to physiological changes in pregnancy and may have complications such as premature delivery, low birth weight, fetal or maternal mortality" [6-8]. Pregnant women in developing countries such as Nigeria are at greater risk due to increasing resistance of bacterial etiological agents of UTI to antibiotics [9,10]. *Escherichia coli* (*E. coli*), a gram negative bacterial belonging to the order enterobacterales has been reported to be the most common pathogen associated with UTI in pregnant women [11-14]. It possess adhesins which has strong affinity to the uroepithelium and mechanisms which enables it to withstand lethal doses of antibiotics such as production of aminoglycoside degrading enzymes, beta lactamase degrading enzymes, alteration of target proteins and overexpression of efflux proteins [4,15]. *Escherichia coli* has also been documented to be a major cause of multi-drug resistance UTI associated with pregnancy in many human populations and experimental models in animals [16]. The present study was designed to determine the prevalence of *E. coli* UTI and its antibiotic susceptibility pattern among pregnant women at the antenatal clinic of the Enugu State University of Science and Technology Teaching Hospital, Enugu, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional and single facility-based study conducted on pregnant women attending antenatal care in the Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu, Nigeria in the period of September to December, 2020. Clean catch "midstream" urine samples were collected from a total of 200 respondents using the convenient sampling technique. Samples were analyzed for bacteriuria and antibiotic susceptibility using standard methods.

2.2 Study Population

This study was carried out in the Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu, Nigeria. The ESUT Teaching Hospital is the major tertiary health facility for the State and is located at the center of the capital city of Enugu for easy accessibility to residents. Enugu State is made up of three senatorial zones namely Enugu East, Enugu West, and North. The senatorial zones are divided into seventeen Local Government Areas comprising 450 communities. The state takes its name from its capital and largest city Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising the Igbo tribe of South Eastern Nigeria; about 50% of which lives in rural areas. It lies between longitudes 6^{o}30'E and 6^{o}55'E and latitudes 5^{o}15'N and 6^{o}15'E and 7^{o}15'E, bordered by Abia State and Imo State to the South, Benue and Kogi States to the North, Ebonyi to the East and Anambra State to the West of Nigeria. "The Enugu State University of Science and Technology Teaching Hospital is accessed by people from different socio-economic backgrounds being located at the center of the city. The hospital serves as referral center for different primary and secondary healthcare facilities in the State, hence its choice for the present study" [17].

2.3 Sample Size

The sample size was determined using the simple proportion method and a prevalence of 15.8% recorded by a recent study among pregnant women in Nigeria was used [18].

$$\mathrm{n} = \frac{Z^2 P(1-p)}{D^2}$$

Where

n = sample size
Z = 1.96 for confidence level at 95%
P = prevalence rate of 15.8%
D = 0.05 for marginal error at 5%.

Substituting

$$\mathrm{n} = \frac{1.96^2 \times 0.158\,(1-0.158)}{0.05^2} = \frac{3.8416 \times 0.158 \times 0.842}{0.0025} = \frac{0.5110710976}{0.0025} = 204.428$$

2.4 Inclusion Criteria

1. Pregnant women with symptoms of UTI who gave informed consent to participate in the study.
2. Pregnant women without symptom of UTI who gave informed consent to participate.
3. Pregnant women that are currently not on antibiotic therapy particularly 2 weeks prior to study.

2.5 Exclusion Criteria

1. Non pregnant women were excluded from the study.
2. Pregnant women who did not give informed consent to participate.
3. Pregnant women who were on antibiotic therapy or who had antibiotic therapy not more than 2 weeks prior to study.

2.6 Sampling Technique

Convenient sampling strategy was used to select participants for the study. Urine sample collection was scheduled for Mondays and Wednesdays in the month of September to December, 2020 until 200 samples were collected. The two days were selected conveniently to make for enough time for urine specimen analysis with emphasis on creating space in the incubator for incubation. The participants were advised on how to collect a "clean catch" mid-stream urine.

2.7 Urine Culture

The urine specimens were cultured on MacConkey Agar and Cysteine Lactose Electrolyte Deficient (CLED) Agar using streaking method. The culture was incubated at 37^{o}C for 24 hours and the plates read. The isolated pathogens were subjected to gram staining and biochemical tests for identification.

2.8 Identification of Isolates

Gram staining and a combination of conventional biochemical testing techniques including Indole test for *Escherichia coli*, catalase and coagulase tests for *staphylococcus aureus*, citrate and Malonate utilization tests for *Klebsiella pneumonia*, oxidase test for *psueudomonas aeruginose*, bile resistance test for *Enterococcus faecalis* and motility test for *Proteus mirabilis* were carried out.

2.9 Antimicrobial Susceptibility Test

"Antimicrobial susceptibility of the isolates was carried out using the modified Kirby-Bauer Disk Diffusion method" [19]. "Muceller Hinton (MH) Agar (Oxoid, UK) was prepared according to the manufacturer's instructions and 10ml transferred into 90mm diameter sterile Petri dishes making a depth of 4mm. The surface was lightly and uniformly inoculated using a sterile cotton wool swab in three directions while rotating the plate to ensure even distribution. Prior to inoculation. The swab stick was dipped into bacteria suspension having visually equivalent turbidity to 0.5 MacFarland standards. Excess liquid from the sterile cotton-wool swab was dipped in the bacterial suspension which was removed by turning the swab stick against the side of the tube. The plates were covered and allowed to dry on the bench before applying the disc. Antibiotics were placed on the agar plate within 15 minutes of inoculation of isolates. The plates were allowed in refrigerator for about 30 minutes for pre-diffusion of the antibiotics once the disks were placed on the medium in order to get more prominent zones (as the lower temperature will curb the growth of the bacterium but not affect the diffusion of the antibiotic) before incubating for 18-24 hours at 37^{o}C. Plates were read on the next day by taking measurement of zone of

inhibition using a meter rue. The *E. coli* ATCC 25922 was used as a negative control. Antibiotic discs used include Ampilicllin (30mg), Ampiclox (30mg), Amoxicillin (30mg), Augumentin (30mg), Tetracycline (30mg), Rocephine (30mg), Nitrofurantion in (30mg) Gentamycin (30mg), Ofloxacin (30mg), Perfloxacin (30mg), Sparfloxacin (30mg), Chloramphenicol (30mg), Efloxacin (30mg), Streptomycin (30mg), Trimethoprim (30mg), Sulfamethaxazole (30mg) and Ceftriazone (30mg) (Oxoid, UK)" [19].

2.10 Data Analysis

Data obtained was analyzed using Statistical Package for Social Sciences Version 23 (IBM Corporations, Armok, NY). Data was presented as descriptive statistics.

3. RESULTS

Out of 200 urine samples collected, 191(95%) samples have bacteria present in them, while 9 samples (4.5%) yielded no significant bacteria growth. Out of the 191 samples that yielded significant bacterial growth, 102 samples (53.4%) were *E. coli*, while the remaining 89 samples (46.6%) revealed significant growth of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus vulgaris* as shown in Table 1. Some of the *E. coli* were resistant to only one drug, while majority were resistant to most of the drugs as shown in Table 2. In general *E. coli* showed resistance rate of 100% to amplicillin, 78% to tetracycline and 70% to Rocephin. Resistance against trimethoprim/sulfamethoxazole and chloramphenicol was observed in the range of 25-60% while it showed low level resistance against nitrofurantoin and ceftriazone 916.7%). It showed 100% susceptibility to ampicillin, streptomycin, septrin and ampiclox. Susceptibility against sparfloxacin, perfloxacin, ofloxacin and efloxacin was observed in the range of 86-98% while nitrofurantoin and ceftriazone shows sensitivity rate of 3.3%.

Table 1. Frequency of Isolated *E. coli* and other organisms in the urine samples

Parameters	Frequency	Percentage
E.Coli	102	53.4%
S. aureus	49	25.6%
K. pneumonia	21	10.9%
P. aeruginosa	10	5.2%
P. vulgaris	9	4.7%
Total	191	100%

Table 2. Antimicrobial susceptibility pattern of *E. coli* isolated from the urine sample

Antimicrobial Agent Tested	Percentage number of *E. coli* Resistant (%)	Percentage number of *E. coli* sensitive (%)
Augmentin (AU)	29	71.0
Ampicillin (AM)	100	0
Gentamycin (CN)	29.7	70.3
Amoxicillin (AM)	33.3	66.7
Septrin (SXT)	0	100
Ofloxacin (OFX)	8.0	92.0
Perfloxacin (PEF)	6.0	94.0
Sparfloxacin (SP)	2.0	98.0
Chloramphenicol (CH)	50.0	50.0
Efloxacin (EF)	14.0	86.0
Ampiclox (APX)	0	100
Ciprofloxacin (CPX)	35	66.7
Tetracycline (TN)	78	21.8
Streptomycin (SN)	0	100
Trimethoprim/Sulfamethoxazole	66.7	30.3
Rocephin	70.8	29.2
Nitrofurantoin	16.7	83.3
Ceftriazone (CTX)	16.7	83.3

4. DISCUSSION

"Pregnant women are at increased risk of developing UTI due to physiological changes during pregnancy. The physiological increase in the plasma volume during pregnancy decreases urine concentration increases the risk of urinary stasis and vesicoureteral reflux. These changes result in difficulty with hygiene due to a distended belly thus increasing the frequency of UTI" [20]. "Additionally, the apparent reduction in immunity of pregnant women appears to encourage the growth of both commensals and non- commensal microorganisms" [21]. "Furthermore, pregnancy-related changes in glomerular filtration rate increases the urinary glucose concentration and alkalinity, thereby facilitating bacterial growth" [22]. "Untreated UTI can adversely affect the health of the fetus or the mother and maybe associated with adverse perinatal outcomes such as premature delivery, low birth weight, premature rupture of ovular membranes and hypertensive syndromes" [23,24]. There are few studies which had investigated the prevalence of multi-drug resistant *E. coli* isolated from the urine samples of pregnant women in Enugu, Nigeria. In the present study, the overall prevalence of multi-drug resistant *E. coli* isolated from the urine samples of pregnant women was 15.4% while 48.5% were symptomatic cases and 5.0% cases were asymptomatic. *Escherichia coli* was the most frequently isolated organism with a prevalence of 53.4% (111 samples) while other pathogens accounted for 46.6% (89 samples). These values are slightly higher to the findings of previous research among pregnant women in the Enugu metropolis which reported an *E. coli* prevalence of 40.6% (89 samples) while other pathogens accounted for 59.4%(295 samples) [25]. Our findings also varies with the findings of some studies which reported an overall prevalence of 25.3% for pregnant women with asymptomatic UTI as against 5.0% recorded in the present study as well as *Klebsiella pneumonia* (45%) reported as the most frequently isolated organism compared to *E. coli* (53.4%) which was the most frequently isolated organism in the present study [26]. The present findings are also higher than the findings of some similar studies conducted at Ibadan Nigeria which reported 8.7% significant bacteriuria among pregnant women but lower to the findings of a study in northern Nigeria which reported 19% prevalence of asymptomatic bacteriuria with *S. aureus* identified as the most frequent isolate (51.1%) and Ondo state, Nigeria which reported prevalence of 74% for significant bacteriuria with S. aureus(23.6%) also identified as the most frequently isolated pathogen.[27,28,29].

We recorded a high resistance of *E. coli* to ampicillin (100%) but a high susceptibility (83.3%) to nitrofurantoin. This is similar to the findings of a recent study in South Africa which reported sensitivity of 81.9% to nitrofurantoin but a resistant of 84.4% to ampicillin/amoxicillin, 55.6% to Trimethoprim/sulfamethoxazole and 50.2% to amoxicillin-clavulanic acid [30]. The use of nitrofurantoin for the management of UTI in pregnancy has been reported to be free from the adverse effects of antibiotics in pregnancy such as cardiovascular or organ-specific malformations, oral cleft or craniosyntosis which are common in treatments with some antibiotics such as cephalosporins, macrolides and quinolones and is supported by the most recent American College of Obstetricians and Gynecologist (ACOG) Committee [30]. This is slightly different from the findings of other studies which also reported a high resistance of *E. coli* to ampicillin (89.5%) but a lower susceptibility (40%) to nitrofurantoin [25]. Variations in the prevalence of multi-drug resistance *E. coli* in different studies may be explained by the fact that differences exist in infrequent prescription, environment, social habits of the community, the standard of personal hygiene, health education practices and policies on the use of antibiotics. In Nigeria, antibiotics are misused more frequently due to lack of a national antibiotic policy for the health system.

5. CONCLUSION

Urinary tract infection due to *E. coli* among pregnant women in Enugu is relatively high and shows high resistance to routine antibiotics; however, nitrofurantoin may be effective against *E. coli* in pregnant women. It is therefore, strongly recommended to undertake a routine culture as part of the antenatal care for pregnant women. A limitation to the present study is the small sample size and the use of a single facility. Further studies involving larger sample sizes and multi-centers are strongly recommended.

6. LIMITATIONS

The limitations of this study were the small sample size, symptoms at time of testing especially as symptoms of UTI and pregnancy are similar and the use of a single center. Future studies in the Enugu population involving a larger sample size and more centers are recommended to address this limitations.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL CONSIDERATION

Ethical approval was obtained from the Research Ethical Review Committee of the Enugu State University of Science and Technology (ESUT) Teaching Hospital, Parklane, Enugu, Nigeria with registration number: ESUT NP/CMAC/RA/034/Vol 3/286). The nature and objectives of the study were duly explained to the subjects before recruitment into the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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