

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

Comment [H1]: Title can be changed in to more précised form

ABSTRACT

Comment [H2]: Divide abstract in to different sections such as background, methodology etc.

Make the abstract more concise and precise.

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. 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 $\geq 10^2$ cfu/ml of urine were regarded as significant bacteriuria. Pure isolates of bacterial pathogen were 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. 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. 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.

Comment [H3]: E.coli induced UTI

Keywords: UTI, *E.coli*, Pregnancy, Enugu

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. 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

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.

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 7,161km² 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°30'E and 6°55'E and latitudes 5°15'N and 6°15'E and 7°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.¹⁶

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.¹⁷

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

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:

$$\begin{aligned}n &= \frac{1.96^2 \times 0.158 (1-0.158)}{0.05^2} \\ &= \frac{3.8416 \times 0.158 \times 0.842}{0.0025} \\ &= \frac{0.5110710976}{0.0025} \\ &= 204.428\end{aligned}$$

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.

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.

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.

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.

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°C for 24 hours and the plates read. The isolated pathogens were subjected to gram staining and biochemical tests for identification.

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 *psuedomonas aeruginose*, bile resistance test for *Enterococcus faecalis* and motility test for *Proteus mirabilis* were carried out.

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°C. Plates were read on the next day by taking measurement of zone of inhibition using a meter rule. The *E.coli* ATCC 25922 was used as a negative control. Antibiotic disc used which includes Ampicillin (30mg), Ampiclox (30mg), Amoxicillin (30mg), Augmentin (30mg), Tetracycline (30mg), Rocephine (30mg), Nitrofurantoin (30mg), Gentamycin (30mg), Ofloxacin (30mg), Perfloracin (30mg), Sparfloxacin (30mg), Chloramphenicol (30mg), Enoxacin (30mg), Streptomycin (30mg), Trimethoprim (30mg), Sulfamethoxazole (30mg) and Ceftriazone (30mg) (Oxoid, UK).

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Data Analysis

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

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 other organisms 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 ampicillin, 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

Comment [H5]: Mention the other organisms

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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%
Other organisms	89	46.6%
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
Septin (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

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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

DISCUSSION

Pregnant women are at increased risk of developing UTI due to physiological changes during pregnancy. Untreated UTI can adversely affect the health of the fetus or the mother.²⁰ 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 this 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).²¹ 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.

We recorded a high resistance of *E. coli* to ampicillin (100%) but a high susceptibility (83.3%) to nitrofurantoin. 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.²³ Variations in the prevalence of multi-drug resistance *E. coli* in different studies

Comment [H9]: Explain about the physiological changes during the pregnancy

may be explained by the fact that differences exist in infrequent prescription, environment, social habits of the community, the standard of personal hygiene and health education practices. In Nigeria, antibiotics are misused more frequently due to lack of a national antibiotic policy for the health system.

Comment [H10]: Add more relevant references which supports the manuscript and compare with the results obtained

CONCLUSION

Urinary tract infection due to E.coli in 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.

Comment [H11]: among

UNDER PEER REVIEW

REFERENCES

1. Johnson JD, Paul CC (2021) Incidence of Escherichia Coli among patients presenting urinary tract infections. *Nigerian Journal of Science and Environment* 19(1):11-15.
2. Begum AM, Jabeeb AM, Lakho R, Zaheen Z, Bala M, Bhutto A. (2022) Prevalence of Urinary tract infection during pregnancy at tertiary care hospital: a cross-sectional study. *Pakistan Journal of Medical and Health Sciences* 16(5):851-853.
3. Nahab HM, Al-Oebady MAH, Munem HAA (2022) Bacteriological Study of urinary tract infections among pregnant women in Al-Samawa City of Iraq. *Archives of Razi Institute* 77(1):117-122.
4. Odongo I, Ssemambo R, Kungu JM (2020) Prevalence of Escherichia Coli and its antimicrobial susceptibility profiles among patients with UTI at Mulago Hospital, Kampala, Uganda. *Interdisciplinary Perspectives on Infectious Disease* 8042540:1-5.
5. Eghbalpour F, Vahdat S, Shahbazi R Mohebi S, Khold S, Hadadi M, Javadi K, Hashemizadeh Z, Motamedfar M (2022) Pathogenic features of urinary Escherichia coli strains causing asymptomatic bacteriuria during pregnancy. *Gene Reports* 27:10559.
6. Peerapur B, Kulkarni S (2018) Prevalence of asymptomatic urinary tract infection due to Escherichia coli among pregnant women attending ANC clinic at BRIMS teaching hospital. *Indian Journal of Microbiology Research* 5(1):84-86.
7. Ali HA, Reda DY, Ormago MD (2022) Prevalence and antimicrobial susceptibility pattern of urinary tract infection among pregnant women attending Hargeisa Group Hospital, Hargeisa, Somaliland. *Scientific Report* 12:1419.
8. Ravi B, Talluri RKR, Sumana K (2022) Bacteria associated with urinary tract infection in pregnant women with overview of their antibiotic susceptibility tests. *International Journal of Current Pharmaceutical Research* 14(4):10-15.

9. Okafor JU, Nweze EI (2022) Antibiotic susceptibility of *Escherichia coli* isolated in cases of urinary tract infections in Nsukka, Nigeria. *Journal of Pre-clinical and clinical Research* 14(1):1-7.
10. Ghaima KK, Khalaf ZS, Abdulhassan AA, Salman NY (2018) Prevalence and antibiotic resistance of bacteria isolated from urinary tract infections of pregnant women in Baghdad hospitals. *Biomedical and Pharmacology Journal*. 11(4):1989-1994.
11. Mohammed JL, Huseyin-Naqid IA, Suer K (2022) Prevalence, antimicrobial susceptibility profile and the associated risk factors of uropathogenic *E. coli* among pregnant women in Zakho City, Kurdistan Region, Iraq. *Women's Health Bulletin* 9(1):1-9.
12. Mishra S, Mohapatra S, Yadav S, Panda GK, Pati P (2018) Prevalence of urinary tract infections in pregnancy and detection of the uropathogenic markers in *E. coli* isolates. *International Journal of Health Sciences and Research* 8(3):48-52.
13. Cotton E, Geraghty R, Umranikar S, Saeed K, Somani B (2022) Prevalence of asymptomatic bacteriuria among pregnant women and changes in antibiotic resistance: a 6-year retrospective study. *Journal of Clinical Urology* 00(0):1-7.
14. Al-Badaii F, Al-Tairi M, Rashid A, Al-Morisi S, Al-Hamari N (2023) Prevalence, risk factors and antibiotic susceptibility of urinary tract infections among pregnant women: a study in Damit District Yemen. *Journal of Pure Applied Microbiology* 17(1):1065-1075.
15. Yeta KI, Mihelo C, Jacobs C (2021) Antimicrobial resistance among pregnant women with urinary tract infections attending antenatal clinic at Levy Mwanewasa University Teaching Hospital (LMUTH), Lusaka, Zambia. *International Journal of Microbiology* 8884297:1-9.
16. Ohotu EO, Queendalyn MN, Ogbuabor AO (2022) Comparative evaluation of platelet indices in preclamptic and non-preclamptic patients in the Enugu Metropolis. *Open Journal of Tropical Medicine* 7(1):001-005.

17. Ali M, Abdoallah MS (2019) Prevalence of urinary tract infection among pregnant women in Kano, Northern Nigeria. *Archives of Reproductive Medicine and Sexual Health* 1(2):23-29.
18. Ugwu LJ, Ogbuabor AO, Anyamene CO (2022) Prevalence of Extended spectrum Beta-lactamase Mediated Urinary Tract Infections in a tertiary hospital in Enugu, South East, Nigeria. *South Asian Journal of Research in Microbiology* 14(1):1-8.
19. Ajuga MU, Otokunefor K, Agbagwa OE (2021) Antibiotic resistance and ESBL production in Escherichia Coli from various sources in Aba metropolis, Nigeria. *Bulletin of the National Research Centre* 45:173.
20. Ranjan A, Sridhar STK, Matta N, Chokkakula S, Ansari KR (2018) Prevalence of UTI among pregnant women and its complications in newborns. *Indian Journal of Pharmacy Practice* 10(1):1-5.
21. Ezugwu IA, Afunwa RA, Onyia FC, Chukwunweijim CR, Offe IM, Onyia CO, Unachukwu MN, Eze AE (2021) Prevalence of urinary tract infections and associated risk factors among pregnant women in Enugu metropolis, Nigeria. *Journal of Biosciences and Medicines* 9:156-171.
22. Patnaik M, Panigrahi K, Das B, Pathi B, Poddar N, Lenka PR, Pattnaik D (2017) Prevalence, risk factors and causative organisms of asymptomatic bacteriuria in pregnancy. *International Journal of Advances in Medicine* 4(5): 1348-1354.
23. Samaga MP (2016) Bacteriological profile of urinary tract infections in pregnant women. *Indian Journal of Microbiology Research* 3(1):17-21.