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SPECTRUM OF BACTERIA CAUSING INFECTION IN PREGNANT WOMEN's URINARY TRACT AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

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Abstract

Background: Urinary tract infections (UTI) rank second among the most common cause of bacteremia among patients in hospitals, women being more vulnerable due to the physiological and structural features of their urethra. It could also involve the lower urinary tract or the bladder.This work seeks to investigate the spectrum of bacteria causing Urinary tract infection amongst pregnant women attending Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FUTHA), and their antimicrobial susceptibility. **Methodology:** Clean voided mid-stream urine samples were collected in sterile universal bottle, and transported to Medical Research Laboratory of AE-FUTHA for analysis.Urinalysis was carried out using Urinalysis strip (combi9).**Results:** Out of the 50 urine samples collected from pregnant woman within the age of 18 to 45 that attended ante-natal at AE-FUTHA, only 32 (64 %) had significant growth of microbes. The organisms isolated were identified as *Escherichia coli (53.13 %), Staphylococcus aureus* (15.13%), *Streptococcus pneumonia* (12.5%), *Klebsiella pneumonia (9.38 %), Enterococcus* sp (6.25%) *and Pseudomonas aeruginosa* (3.13%). All the isolates were susceptible to Meropenem, Amikacin, Gentamycin and Ciprofloxacin with overall sensitivities of (93.2%), (84.6%), (83.0%) and (78.1%) respectively. Isolates were mostly resistant to Ceftazidime, Penicillin, Azithromycin, nitrofurantoin and Erythromycin with overall resistance rates of (89.7%), (86.5%), (78.5%), (76.2%), and (66.6%) respectively. **Conclusion:** The result revealed that *E. coli* had the most frequency of occurrence, and still remains the major cause of UTI in pregnant women within the ages of 18-35 years. It is recommended that the use of Meropenem, amikacin, gentamycin and Ciprofloxacin should be considered as first line regimen for UTI treatment.

Keywords: Susceptibility, spectrum, bacteria, antimicrobial, pregnant women.

1.0 Introduction

Urinary tract Infections (UTIs) are the second most common cause of bacteremia in hospitalized patients and women are more vulnerable to UTIs due to the physiological and structural features of the female urethra (Kawser *et al*., 2011). It may involve the lower urinary tract or the bladder (Erica *et al*., 2013). In the female human subject, the urinary tract has an important relationship with the reproductive organs because of its proximity. The uterus surrounds and partially covers the bladder when a woman is not pregnant. When a woman is pregnant, the uterus grows and at

various points throughout the pregnancy, all the tissues in the urinary system are affected. UTI in pregnant women if left untreated can adversely affect the pregnant woman and the infant (Manjula *et al*., 2013).

Due to its anatomical connection to the vagina, a woman's urethra is susceptible to harm during sexual activity and to simple microbial entry into the bladder during pregnancy and childbirth. According to Behzadi *et al*. (2010), there were variations in the epidemiology, species distribution, and susceptibility patterns of uropathogens among the population and sites evaluated. Enteric bacteria, especially *Escherichia coli*, continue to be the most common cause of UTIs despite changes in the distribution of pathogens that cause UTIs. These bacteria also produce a significant amount of workload in clinical microbiology laboratories. Sex, age, disease, hospitalization, and restriction of the flow of urine are a few of the anomalies and UTI variables that affect the body's natural ability to fight infections. UTIs cause a lot of morbidity and, when combined with renal papillary injury or urinary blockage, can seriously harm the kidneys. The prevalence of resistant bacteria has been rising on a global scale. Currently, Grams negative bacteria like *Klebsiella pneumonia and E. coli* have high prevalence of resistance in several nations (Michael *et al.*, 2020). One of the greatest and most extensively researched free-living organisms is the bacteria *Escherichia coli*, which is also a major cause of urinary tract infections. The remarkable diversity of this species is further demonstrated by the fact that some strains of Escherichia coli are harmless commensals in the intestines of animals, while other genotypes, such as enteroaggregative, enterohemorrhagic, enteroinvasive, enteropathogenic, and enterotoxigenic, *E. coli*, cause significant mortality and morbidity as human intestinal pathogens. The goal of this study was to identify the bacteria at Alex Ekwueme Teaching Hospital that cause urinary tract infections in pregnant patients, as well as their susceptibility to antimicrobial medications.

2.0 Materials and methods

2.1 Study Area

The Alex Ekwueme Federal University Teaching Hospital in Abakaliki served as the study's location. Over 1.1 million people receive medical care from this largest hospital in Abakaliki, which also serves as a teaching and referral facility. Its 7235.3 km^2 territory is situated between longitudes 6.3231^oN and latitudes 8.1121^oE. Agriculture and Mining is the mainstay of Abakaliki, Ebonyi state economy. The state's weather is conducive for large-scale farming, and mining. They produce rice majorly and is consumed locally and sold to consumers in neighboring towns and cities. The livelihoods in the Abakaliki also depend on employment and business. This hospital serves other states like Enugu, Abia, Benue, Imo, and Cross River among others.

2.2 Study population

The study targeted both out-patients and in-patient pregnant women between the age of 18 to 35 presenting with symptoms and signs of UTI such as: dysuria, fever, nausea, and flank pain. Baseline demographic data including age, sex, level of education and risk factors such as catheterization, history of UTI, also out and inpatients were also collected.

Figure 1: Map showing the Study Area (Abakaliki) in Ebonyi State.

2.3 Sample collection

Midstream urine samples were collected in sterile sample bottles from 50 pregnant women (Table 1). The samples collected were labelled accordingly and taken to the laboratory (Medical research laboratory AE-FUTHA). All specimens were analyzed within 12hrs of sample collection to avoid contamination or deterioration of leucocytes. Processing of the specimen was done to meet the required number which is ten (10) set for the day (Onifade *et al.*, 2011). Samples were collected between December 2022 and January 2023.

Table 1: Total number of samples collected

Sample by Age range	Number of samples
18 – 23	3
24 – 29	7
30 – 35	20
36 – 41	15
41 – 45	5
Total	50

2.4 Isolation and Identification of Uropathogens

Isolation and identification of the bacterial uropathogens was done by inoculating uniformly mixed midstream urine sample on Cysteine Lactose Electrolyte Deficient (CLED) Agar, incubated at 37^{o}C for 24 hrs. (Odoki *et al*., 2019). Colonies that develop on the medium after 24 hours of incubation were counted and recorded in colony forming unit per milliliter (CFU/mL). Thereafter the colonies were sub-cultured into MacConkey and Blood agar. Agar plates were

incubated at 37^{o}C for 18hrs. Colonies that grow on the sub-cultured agar plates were identified further by biochemical characteristics, size, colonial morphology and characteristics on CLED, MacConkey and Blood agar

2.5 Urinalysis

Urinalysis was carried out using Urinalysis strip (combi9). Proper care in handling the samples were employed. This was done by dipping each strip into the urine sample and color changes was matched with various color code as provided by the manufacturers. Appropriate readings were done and results recorded.

2.6 Microscopic examination

Three milliliter (3 mL) of urine sample were collected using a 5 mL syringe and balanced with 3 mL of water in a test tube placed in centrifuge for 5mins at 3500rpm, clear supernatant were removed and sediment were collected, placed in a clean glass slide and viewed under the microscope at 10x magnification.

2.7 Antibiotic Susceptibility Test

The Kirby Bauer disc diffusion method was used to test the antibiotic susceptibility of isolated bacteria. One milliliter of pure broth cultures was transferred into a micro-centrifuge tube containing two milliliters of 0.85% physiological sterile saline. The mixture was gently vortexed, and the turbidity of the resulting inocula was adjusted to 0.5 McFarland standard solution. One hundred microliters (1µL) of the suspension was evenly spread on Mueller Hinton agar plates, which were then left to dry for ten minutes before the antibiotic discs were added. The seeded plates were allowed to sit on the bench for thirty minutes the antibiotic to properly diffuse. Finally, the plates were incubated in an inverted position at 37 °C for a 24 hrs. After incubation, the zones of inhibition were observed, recorded and interpreted following the recommendations of Clinical and Laboratory Standards Institute (CLSI, 2016).

2.8 Data Analysis

The data was analyzed using Pearson's Chi-square ($\chi 2$) test at 95% confidence interval (CI). Four age groups with a 10-year difference were created by stratifying the demographic data using percentages and frequencies. The significance difference between the isolated bacterial pathogen with various age groups, and statistical comparisons for sensitivity pattern were determined using the Chi square test. The Le and Boen *et al*. (1995) formula was used to calculate the incidence of bacteriuria.

3.0 Results

The appearance of the urine as physically observed, revealed most of which were pale yellow followed by Amber and clear, then deep amber, and Turbid. 30 (60%) were pale yellow, while 10(20%), 6(12%) and 4(8%) were amber, and turbid respectively (Table 2).

Table 2: Appearance of Urine Samples

Appearance	Total No. of urine
Pale yellow	30 ± 0.00^{a}
Amber and clear	10 ± 0.10^{ab}
Deep amber and clear	6 ± 0.00^{a}
Turbid	4 ± 0.01^{b}

Legend = ± Se=Standard error; the attached letters signifies significant difference ($p<0.05$). Numbers with same letters within rows are not significantly different.

The bacterial count showed that samples from ages 41 to 45 had the highest bacterial count of $32x10^5$ CFU/mL, while ages 36-41 had the lowest bacterial count of $4\ x10^5$CFU/mL (Table 3).

Table 3: Enumeration of bacterial growth on Cysteine Lactose Electrolyte Deficient (CLED) Agar

Sample per Age range	**Bacterial count CFU/mL**
18 – 23	**5** $x10^5$
24 – 29	14 $x10^5$
30 – 35	9 $x10^5$
36 – 41	4 $x10^5$
41 – 45	32 $x10^5$

The parameters and outcome of the urinalysis strip shows the possibility of samples having significant growth when cultured and possible signs of infections.Samples with positive bacteremia had a pH range of 6.0 to 8.0 while those that were negative had pH 4.0 to 5.5 (Table 4).

Table 4: Reactions of Urine samples to different parameters on the combin 9 urinalysis strip.

Parameters	Positive	Negative
pH	40 (6.0 – 8.0)	10 (4.0 – 5.5)
Glucose	15 ± 0.00^{a}	35 ± 0.50^{ab}
Ketone	5 ± 0.05^{ab}	45 ± 2.50^{c}
Nitrite	12 ± 0.00^{a}	38 ± 1.02^{ab}
Bilirubin	0 ± 0.10^{a}	50 ± 1.81^{ab}
Blood	7 ± 0.11^{abc}	43 ± 2.00^{bc}
Protein	10 ± 1.00^{b}	40 ± 1.00^{ab}

Legend: ± =Standard error; the attached letters signifies significant difference ($p<0.05$). Numbers having same letters in rows are not significantly different.

The presence of Blood were observed in 5, 4, and 1, samples from ages 30-35, 36-41 and 24-29 respectively. Five (5) Out of the 20 samples from ages 30-35 of the sample had visible blood present in the urine, 15 were without blood, 3 positive protein and 17 samples showed absence of protein (Table 5).

Table 5: Age Distribution of pregnant women with UTI.

Age range	Blood		Protein		Nitrite		GN	GP
	+	-	+	-	+	-		
18 – 23	0	3	1	0	0	3	0	0
24 – 29	1	6	2	5	0	7	2	3
30 – 35	5	15	3	17	4	16	11	3
36 – 41	4	11	2	13	3	12	7	2
41 – 45	1	4	3	2	1	4	2	2
Total	11	39	11	37	8	42	22	10

Legend: += Positive, -= Negative, CFU; Colony Forming Unit, ml; Milliliter, GP; Gram positive, GN; Gram negative.

Out of the 50 (100%) urine samples collected 32 (64%) had significant bacterial growth, Twenty one 21 (42%) of the bacteria were isolated to be Gram negative bacteria and Eleven 11(22%) of the isolates were Gram positive, out of the 32 (64%) isolates, six were *E. coli*, *Streptococcus* sp, *Klebsiella* sp, *S. aureus, Pseudomonas aeruginosa* and *Enterococcus sp.* were isolated and identified by biochemical characteristics, colonial appearance on CLED, Macconkey and Blood agar (Table 6).

Table 6: Identification of bacteria using Biochemical Test

Biochemical test					**Probable isolates**
Urease	Citrate	Indole	Oxidase	Coagulase	
-	-	+	-	-	*Escherichia coli*
-	-	-	-	-	*S. aureus*
-	-	-	-	+	*Streptococcus sp*
-	-	-	+	-	*P. aeruginosa*
+	+	-	-	-	*Enterococcus* sp.
-	-	-	-	-	*Klebsiella sp*

Legend: + =Positive reaction, - =Negative reaction.

The results also showed *E. coli* as the most frequently isolated bacterial pathogen with 17 (53.13%, $\chi 2$ =2.173, P value 0.001) causing bacteriuria in pregnant women attending Alex Ekwueme Federal Teaching Hospital Abakaliki (Table 7). The second most frequent pathogen was *Staphylococcus aureus* with 5 (15.13%), the third most frequent bacterial isolate was *Streptococcus* sp with 4 (12.5%), *Klebsiella* sp was fourth with 3 (9.38%) and *Enterococcus* sp the fifth with 2 (6.25%) of the bacterial isolates and *Pseudomonas aeruginosa* been the least with 1 (3.13%) (Table 7).

Table 7: Frequency of occurrence of Isolates

ISOLATES	Frequency of occurrence	Percentage Frequency of occurrence (%)
Escherichia coli	17	53.13 ± 0.00^{a}
Staphylococcus aureus	5	15.13 ± 0.01^{ab}
Pseudomonas aeruginosae	1	3.13 ± 0.21^{ab}
Streptococcus pneumonia	4	12.5 ± 0.10^{b}
Enterococcus sp	2	6.25 ± 0.00^{a}
Klebsiella pneumonia	3	9.38 ± 0.01^{ab}

Legend ± =Standard error; the attached letters signifies significant difference ($p<0.05$). Numbers bearing same letters within rows are not significantly different.

In general the antibiotics with the highest susceptibility rates were Meropenem, Gentamycin and Ciprofloxacin (CIP) with susceptibility rates of 82.4%, 70.6% and 58.8% respectively among the *E.coli* species isolated. Overall, these results highlighted the importance of the use of Meropenem in the treatment of bacteriuria associated with *E.coli* (Figure 2).

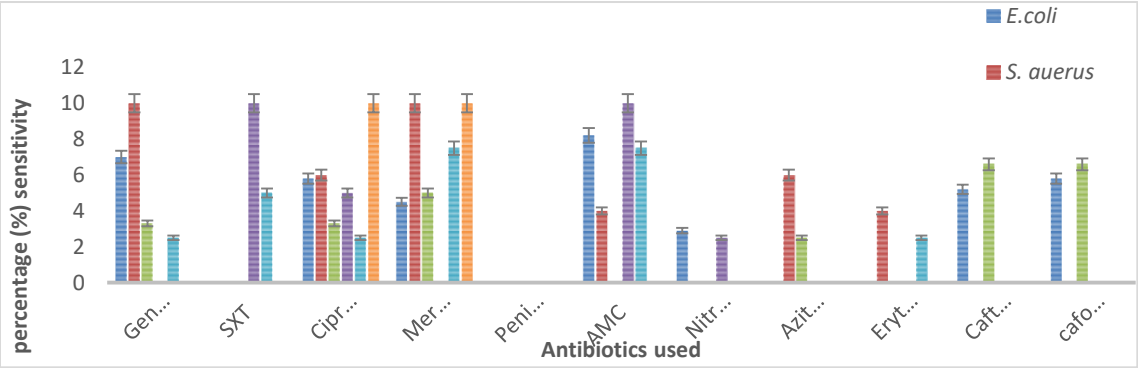

Figure 2: Antibiotic sensitivity profile to isolated bacterial pathogens among pregnant women attending AE-FUTHA

Legend: SXT= Sulfamethoxazole-trimethoprim, AMC= Amoxicillin with clavulanic acid, *E.coli = Escherichia coli, S. aureus = Staphylococcus aureus, K. pneumonia = Klebsiella pneumonia, Enterococcus. feacalis = Enterococcus feacalis, S. pnuemonia = Streptococcus pneumonia, P. aeruginosa = Pseudomonas aeruginosa*

Discussion

In this study, bacteriuria was high (81%) in age group 30-35 years, with *E. coli* being the most frequently occurred and isolated organisms. This is in agreement with previous studies by Mwaka *et al.,* (2021) which reported *Escherichia coli* with high prevalence of 57.5% within ages 35years. Studies by Mohammed *et al*. (2023) and Chennu *et al.* (2022) also reported high prevalence (62%) of bacteriuria caused by *E. coli*. This prevalence is a little lower compared to this study possibly due to study population involved. The second most isolated pathogen was *S. aureus* with 30.5% rate of bacteriuria isolation of *Staphylococcus aureus* in this study could be associated with the bacterium having the ability for choosy adherence to human urothelium where it causes direct hemagglutination. Previous research conducted by Mwaka *et al*. (2021) in Mulago Hospital, Uganda, revealed a prevalence of 24.9% for *S. aureus*. This result, however, differs from that of a 2016 study conducted in Nepal by Singh *et al*., which found that 8.3% of people had S. aureus. The study environment and the individuals involved in the study may have contributed to the variation. Nevertheless, the significant variation in prevalence could be attributed to the study's time and geographic area (Okorondu *et al*., 2023).

The determined bacterial pathogens had overall sensitivity values of 93.2%, 84.6%, 83.0%, and 78.1% to ciprofloxacin, gentamycin, amikacin, and meropenem, respectively. The overall resistance rates of the identified bacterial pathogens to Ceftazidime, Penicilin, Azithromycin, Nitrofurantoin, and Erythromycin were (89.7%), (86.5%), (78.5%), (76.2%), and (66.6%), respectively. This drug sensitivity pattern is at odds with the results of Ezechi et al. (2020), who found that Nitrofurantoin exhibited the strongest and broadest range of efficacy against the microorganisms that cause UTIs. Additionally, it differs from the pattern of microorganism sensitivity found in the urine of women attending general public hospital in Ilorin, as reported by Akanbi *et al.* (2020), wherein third-generation Cephalosporins and Floroquinolones demonstrated the greatest efficacy against uropathogens. However, the current study's results showed a lower prevalence than other cases that have been reported. This could be explained by the potential that some of the women in our sample were taking antibiotics at the time of sample collection (Ekwealor *et al*., 2016).

Conclusion

E. coli and *S. aureus* were the most commonly identified microorganisms in this study. We found increased antibiotic resistance in *Enterobacter* species. The overall resistance rate of the identified bacterial pathogens to Ceftazidime, Penicilin, Azithromycin, Nitrofurantoin, and Erythromycin were above 50%. Hence, the finding of this study however calls for the use of Meropenem, Amikacin, and Ciprofloxacin as first line regimen for UTI treatment.

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Author's Contribution.

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Provision of funds: Victor-Ekwebelem Munachimso Odenakachi and Okorie Michael Chikodiri

Performance of experiment: Victor-Ekwebelem Munachimso Odenakachi, Okorie Michael Chikodiri, and Chidiebere Ene Brown

Data analysis and manuscript drafting: Victor-Ekwebelem Munachimso Odenakachi, Okorie Michael Chikodiri and Simeon Okiki Naphtali

Proof reading of the manuscript: All Authors

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