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Etiology, Prevalence And Antimicrobial Susceptibility Profile Of Urinary Tract Infection Among Pregnant Women In Enugu, South East Nigeria

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Citation

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Abstract

Background: Urinary tract infections (UTIs) are the most prevalent bacterial infections in pregnancy.

Objective: To determine the prevalence of UTIs, aetiological agents and antimicrobial susceptibility profile of the isolates among pregnant women attending antenatal clinics in some selected hospitals in Enugu.

Materials and Methods: A cross-sectional study was carried out between February and September 2018 in the Microbiology laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla Enugu, Nigeria (UNTH). A total of 420 clean-catch midstream single urine samples were collected and analyzed using the standard microbiological procedure. Information on age, gestational age, gravidity, level of education and area of residence were recorded in a well-structured questionnaire. Bacterial isolates were identified based on their colonial morphology, Gram staining and biochemical reactions. Antimicrobial susceptibility profile was determined using disc diffusion techniques. Statistical analysis was done using GraphPad Prism version 7.0 and p-values ≤0.05 was considered to be statistically significant.

Results: Of the 420 samples, 96 yielded significant bacterial growth giving an overall prevalence of 22.9%. Gram-negative organisms accounted for 64.5% of the isolates and Gram-positive 35.5%. The bacterial pathogens isolated were Escherichia coli 50%, Staphylococcus aureus 18.8%, Staphylococcus saprophyticus 10.4%, Klebsiella spp 8.3%. Others were Enterococcus fecalis 6.3%, Pseudomonas aeruginosa 4.2% and the least was Citrobacter spp 2.0%. Antimicrobial susceptibility profile showed that the majority of the isolates were sensitive to Imipenem and Nitrofurantoin while most were significantly resistant to, augmentin, ampicillin, cotrimoxazole, gentamicin, cefuroxime, cefotaxime, and ceftazidime.

Conclusion: Prevalence of urinary tract infection was high in this study. Routine screening of pregnant women is imperative to avoid pregnancy complications. Increasing antibiotic resistance to commonly used antibiotics is a challenge to the health-care system. Inappropriate use of antibiotics must be discouraged. Regular antibiotic susceptibility surveillance in addition to antibiotic stewardship policy should be adopted.

INTRODUCTION

Urinary tract infections are the most prevalent bacterial infections during pregnancy [1] The burden of UTI disease is estimated at 150 million cases yearly worldwide, with various types of UTI causing significant health problems that affect millions of people [2]. The rate of morbidity and financial burden is high.[3] Urinary tract infections are prevalent in women than in men and, far more common in pregnant women than non-pregnant women [1]. The magnitude of UTI depends upon both the bacteria's virulence and the host's susceptibility. The array of UTI diseases includes; cystitis, pyelonephritis, urosepsis, catheter-related infection and asymptomatic bacteriuria [4]. Signs and symptoms include painful urination or burning sensation, dysuria, desire to urinate always, blood or mucus in the urine, cramps and lower abdominal pain, discomfort during sex, pressure and, sensitivity in the bladder and when the bacteria migrate to the kidneys, patients experience back pain, chills, fever, nausea, and vomiting [5, 6]. It has been reported that there is a higher risk for UTI, starting from 6th week and the peak rates from 22nd to 24th weeks [7, 8]. The increased risk of developing UTI during pregnancy is predominantly attributed to the previous history of UTIs and other risk factors such as lower social class, basic hygiene, sickle cell trait, anemia, increased parity or age, number of childbirths, number of intercourse per week and lack of antenatal care [9]. Abnormalities in the urinary tract and diabetes mellitus may also predispose to UTIs during pregnancy [10, 11]. The pressure of the uterus on ureter may cause stasis of urine due to humoral and immunological changes during normal pregnancy and this may increase the risk of UTI [12]. Changes in urine chemistry with elevated levels of glucose and amino acid encourage bacterial growth [13].

Urinary tract infections are mostly caused by Gram-negative organisms than Gram-positive organisms. Gram-negative organisms include E. coli, Klebsiella spp, Proteus spp, and Pseudomonas spp. Gram-positive organisms include Streptococcus species, Staphylococcus species and Enterococcus species [14, 8, 15]. These organisms are predominantly from the external genitalia, vagina, rectum, and gastrointestinal tract [16]. Urinary tract infections in pregnancy may be categorized into asymptomatic and symptomatic. Asymptomatic occurs due to anatomical and physiological changes and it involves the lower part of the urinary tract resulting in asymptomatic bacteriuria. Symptomatic involves the upper part of the urinary tract leading to symptomatic bacteriuria and it is characterized by acute pyelonephritis which is the most common cause of predelivery hospitalization [17] Pregnancy increases the continuity from asymptomatic to symptomatic bacteriuria which may lead to acute pyelonephritis in 20-50% of cases promoting unfavorable obstetric outcomes like prematurity, anemia, UTIs and higher fetal mortality rates if not treated [18].

UTI is generally handled with broad-spectrum antibiotics, and the therapy commences empirically without carrying out laboratory culture and sensitivity. This improper and non-judicious use of antibiotics has led to the development of global antibiotic resistance in bacteria, resulting in the emergence of multi-resistant strains of pathogenic bacteria [19]. The prevalent uropathogens are been found to be resistant to most chemotherapeutic agents and the development of this resistance will limit treatment options. Adequate treatment and control of these conditions need a good understanding of bacteria species involved and their susceptibility to various antibiotics [20]. The antibiotic susceptibility profile in bacteria differs from country to country. As a result of the increasing prevalence of antibiotic-resistant urinary pathogens, the use of most appropriate antibiotics must be adopted for the first choice empiric therapy of asymptomatic UTI. Worthy to note is the need to circumvent antibiotics known to have a high rate of antimicrobial resistance [19].

MATERIALS AND METHODS

Study design and subjects

It was a cross-sectional study on 420 pregnant women attending antenatal in one big private hospital, Balm of Gilead Hospital, Mary-land, and one tertiary institution, University of Nigeria Teaching Hospital, Ituku Ozalla all in Enugu metropolis to get a well-distributed population. People travel from far and near to attend these too hospitals because of their peculiarities. A standard questionnaire was used to record bio-data and demographic variables.

Sample Size

The method used by Uddin and Khan was used to obtain a sample size [1]. The UTI prevalence of 21% from a previous study was used at a 95% confidence interval (CI) and a 5% margin of error. A sample size of 255 was required; however, because of the high turnout of patients, 420 pregnant women were enrolled.

Ethical Clearance

This work was approved by the Ethics Committee of the University of Nigeria Teaching Hospital, Ituku Ozalla Enugu.

Inclusion Criteria

Pregnant women who gave their consent and who is resident within the study area and at any gestational age were enrolled. Each participant was recruited once.

Exclusion Criteria

Women that had symptoms of urinary tract infection or fever or had taken antibiotics within 2 weeks of the study or had other chronic or debilitating conditions and those that did not give their consent were not included in the study.

Sample/ Data Collection

The women who met the inclusion criteria were briefed on how to collect midstream urine. They were provided with a wide-mouthed sterile universal container for sample collection. The subjects filled their questionnaires and submitted their well-labeled samples that were coded in line with the questionnaires. The urine samples were taken to the laboratory for analysis.

Laboratory Diagnostic Methods

Urine samples were cultured on Cystine lysine electrolyte deficient (CLED) agar and blood agar using sterile, calibrated standard wire loop (0.002ml). The plates were incubated at 37o C for 24-48 hours and observed for significant growth. Colony counts were determined at the end of the incubation period, urine samples with growth that had over 105 CFU per milliliter were reported as significant [21]. The isolates were identified based on colonial morphology, Gram stain and standard biochemical tests. The isolates were subjected to antimicrobial susceptibility test using Kirby-Bauer disc diffusion as per Clinical Laboratory Standard Institute (CLSI) criteria on Mueller-Hinton agar (MHA) [22]. The following antibiotics were used Augmentin(µg), Ampicillin(30µg), Cefuroxime(30µg), Cefoxitin(30µg), Cefotaxime(30µg), Ceftazidime(30µg), Imipenem(10µg), Amikacin(30µg), Gentamicin(10µg), Ofloxacin(5 µg), Ciprofloxacin(5µg), Nitrofurantoin(300µg), and Cotrimoxazole(25 µg)

Detection for MBL production

The pathogens that were resistant for imipenem were screen for MBL production using Imipenem+EDTA. The test organisms were inoculated on Muller-Hinton agar. A preparation of 0.5M EDTA was made. Two 10µg imipenem discs were placed on the surface of the agar plate and EDTA solution was added to one of the discs to obtain a needed concentration of 750 µg. A comparison of the two discs was done after 18hrs of incubation at 37o C. Zone enhancement with EDTA impregnated imipenem was considered to be positive. Enhancement in the zone of ≥7mm in imipenem EDTA disc, when compared to only imipenem, was taken as positive for MBL production [23].

Stains of Staphylococcus aureus that were resistant to cefoxitin were reported as resistant to all B-lactam antibiotics including penicillins, cephalosporins, and carbapenems and were, therefore, methicilin resistant [24, 22].

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism version 7.0. Values were expressed as Mean± S.E. Student T-test and One-way Analysis of Variance (ANOVA) were used for comparison of frequencies between groups at a 95% confidence interval. A P-value of <0.05 was considered statistically significant.

RESULTS

A total of 420 midstream urine samples were collected from pregnant women who met the inclusion criteria. Ninety-six single isolates were recovered giving a prevalence of 22.9%. Table 1 shows that Escherichia coli had the highest percentage of occurrence (50%), followed by Staphylococcus aureus (18.8%), Staphylococcus saprophyticus (10.4%), Klebsilla spp (8.3%), Enterococcus spp (6.3%), Pseudomonas aeruginosa (4.2%) and the least was Citrobacter spp (2.0%). Table 2 shows the demographic variables of the study population and the distribution of bacteriuria among pregnant women. Age, gestational age, gravidity, and level of education had no significant association with the presence of bacteriuria P>0.05. Table 3 shows the susceptibility and resistance profiles of isolates. Most of the isolates showed resistance to most of the commonly used antibiotics, augmentin, ampicillin, gentamicin and cotrimoxazole. Imipenem had the lowest resistance to all isolates 2.7% followed by nitrofurantoin 23.8%, and ciprofloxacin 34.3%. Pseudomonas aeruginosa isolates from this study had resistance to almost all the antimicrobial tested except imipenem. Only one isolate of E. coli was resistant to imipenem. Only one isolate of E. coli was found to be imipenem resistant and the test for MBL showed that it was negative. Six isolates of Staphylococcus aureus were found to be methicillin-resistant.

Table 1

Frequency Distribution of isolated Bacteria from Pregnant Women

Bacterial Isolates	**Number Isolated (%)**
Escherichia coli	48(50)
Klebsiella spp	8(8.3)
Pseudomonas aeruginosa	4(4.2)
Citrobacter spp	2(2.0)
Staphylococcus aureus	18(18.8)
Staphylococcus saprophyticus	10(10.4)
Enterococcus spp	6(6.3)
Total	96(100)

Table 2

Demographic variables of the study population and prevalence of bacteriuria among pregnant women

Demographic variables	Number Examined	Number Positive	% Positive	P-value
Age Group				
21-25	100	20	20.8	0.196
26-30	150	46	47.9	
31-35	100	24	25.0	
36-40	70	6	6.3	
Gestational Age				
First Trimester	98	12	12.5	0.29
Second Trimester	150	38	39.6	
Third Trimester	172	46	47.9	
Gravidity				
Primigravidae	99	14	14.5	0.24
Secundigravidae	121	28	29.2	
Multigravidae	200	54	56.30	
Level of Education				
Primary	6	0	0	0.15
Secondary	219	38	39.6	
Tertiary	195	58	60.4	
Total	420	96	22.9	

Table 3

Sensitivity Pattern of Bacterial Isolates from Pregnant Women

Antibiotics			Bacterial Isolates						%Mean
		E. coli	Klebsiella spp	Pseudomonas aeruginosa	Citrobacter spp	Staph. aureus	Staph. saprophyticus	Enterococcus	
Augmentin	S	16(33.3)	4(50.0)	0(0.0)	0(0.0)	2(11.1)	2(20.0)	0(0.0)	16.3
	R	32(66.7)	4(50.0)	4(100)	2(100)	16(88.9)	8(80.0)	6(100)	86.7
Ampicillin	S	14(21.2)	0(0.0)	0(0.0)	0(0.0)	10(55.6)	5(50.0)	1(16.7)	20.5
	R	34(70.8)	8(100)	4(0.0)	2(100)	8(44.5)	5(50.0)	5(83.3)	79.5
Cefuroxime	S	10(20.8)	0(0.0)	0(0.0)	0(0.0)	10(55.6)	7(70.0)	0(0.0)	20.9
	R	38(79.2)	8(100)	4(100)	2(100)	8(44.5)	3(30.0)	6(100)	79.1
Cefoxitin	S	24(50)	5(62.5)	0(0.0)	0(0.0)	12(66.7)	8(80)	1(16.7)	39.4
	R	24(50)	3(37.5)	4(100)	2(100)	6(33.3)	2(20)	5(83.3)	60.6
Cefotaxime	S	4(8.3)	0(0.0)	0(0.0)	1(50.0)	8(44.5)	8(80.0)	0(0.0)	26.1
	R	44(91.7)	8(100)	4(100)	1(50.0)	10(55.6)	2(20.0)	6(100)	73.9
Ceftazidime	S	6(12.5)	0(0.00)	0(0.0)	1(50.0)	8(44.5)	8(80.0	2(33.7)	31.5
	R	42(87.5)	8(100)	4(100)	1(50.0)	1055.6)	2(20.0)	4(66.7)	68.5
Imipenem	S	47(97.9)	8(100)	4(100)	2(100)	15(83.3)	10(100)	6(0.0)	97.3
	R	1(2.1)	0(0.0)	0(0.0)	0(0.0	3(18.8)	0(0.0)	0(0.0)	2.7
Amikacin	S	20(41.7)	4(50.0)	1(25.0)	1(50.0)	7(38.9)	7(70.0)	1(16.7)	41.8
	R	28(58.3)	4(50.0)	3(75.0)	1(50.0)	11(61.1)	3(30.0)	5(83.3)	58.2
Gentamicin	S	21(43.8)	2(25.0)	0(0.0)	1(50.0)	2(11.1)	8(80.0)	0(0.0)	29.9
	R	27(56.3)	6(75.0)	4(100)	1(50.0)	16(88.9)	2(20.0)	6(100)	70.1
Ofloxacin	S	32(66.7)	6(75.0)	0(0.0)	0(0.0)	6(33.3)	6(60.0)	2(33.3)	38.4
	R	16(33.3)	2(25.0)	4(100)	2(100)	12(66.7)	4(40.0)	4(66.7)	61.6
Ciprofloxacin	S	36(75.0)	5(62.5)	0(0.0)	2(100)	10(55.6)	10(100)	2(33.3)	65.7
	R	12(25.0)	3(37.5)	4(100)	0(0.0)	8(44.5)	0(0.0)	4(66.7)	34.3
Nitrofurantoin	S	48(100)	8(100	0(0.0)	2(100)	12(66.7)	10(100)	4(66.7)	76.2
	R	0(0.0)	0(0.0)	4(100)	0(0.0)	6(33.3)	0(0.0)	2(33.3)	23.8
Cotrimoxazole	S	14(29.2)	3(37.5)	0(0.0)	0(0.0)	8(44.5)	5(50.0)	2(33.3)	27.8
	R	34(70.8)	5(62.5)	4(100)	2(100)	10(55.6)	5(50.0)	4(66.7)	72.2

DISCUSSION

Urine culture remains the gold standard method for screening pregnant women for urinary tract infections during pregnancy. In this study, we recorded a prevalence of 22.9% and this was consistent with two studies done in Kenya and Benin Nigeria which reported prevalence of 21.5%, 26.7% and 21% respectively [25, 16, 26]. Our result was however higher than studies done in Southwest Nigeria (10.5%), China (15%), Sudan (14.7%), and Bangladesh (14.6%) [27, 28, 1]. Notwithstanding, other studies had reported a higher prevalence in India (35%) and Uganda (32.2%) [17, 29] and much higher prevalence in Afikpo (55%) and Nasarawa (62.7%) Nigeria [30, 31]. The prevalence of asymptomatic bacteriuria in pregnant women differs from geographical region to another even within the same country as seen in this work [26, 30, 31].The variability in prevalence may be explained by the differences in the community's climate, social habits, personal hygiene practices, and education [16]. In pregnancy, there exists a relationship between asymptomatic UTI and symptomatic UTI. Studies that were done in Kenya reported different prevalence rates in the same country but different localities (21.5%) in Nairobi and 19.7% in Gucha [25, 32]. Pregnancy increases the succession from asymptomatic to symptomatic bacteriuria which may lead to acute pyelonephritis in 20-50% of cases promoting unfavorable obstetric outcomes like prematurity, anemia, UTIs and higher fetal mortality rates if left untreated [18].

Many risk factors have been reported to predispose pregnant women to asymptomatic bacteriuria. However in this analysis, maternal age, gestational age, gravidity and level of education of subjects had no statistically significant effect on urinary tract infection. The age distribution of subjects in this research seemed not to have any significant effect on UTI. This aligned with the study done in Kenya [16] and Tanzania [33].

The uropathogens isolated from this study were Escherichia coli (50%), Staphylococcus aureus (18.8%), Staphylococcus saprophyticus (10.4%), Klebsiella spp (8.3%), Enterococcus fecalis (6.3%), Pseudomonas aeruginosa (4.2%). and Citrobacter spp (2.0%). Escherichia coli was the most dominant pathogen isolated in this study and this was consistent with several studies done in different parts of the world [25, 30, 26, 27, 1, 16, 31]. Staphylococcus aureus ranked second (18.8%), comparable results were reported in different parts of the world [25, 16, 27] but at variance with Onuoha et al., Ajide et al, Uddin and Khan [30, 31,1] that reported, Klebsiella spp, while Mordi et al and Ranjan et al., recorded Enterococcus [26, 17]. Gram-negative organisms were more prevalent (64.6%) than Gram-positive bacteria (35.3%). This was comparable with studies done in Kenya that reported Gram-negative (55.3%), and Gram-positive

(44.71%) and Tanzania Gram-negative (61.9%) and Gram-positive bacteria (38.1%)[25,34] and many researchers reported same. E. coli remains the most predominant pathogen in UTI because of the number of virulence factors known for its colonization and invasion of the urinary epithelium. The Gram-negative bacteria in general have unique structures that enable them to attach to the uro-epithelium and prevent them from being flushed away by urine [25].

Treatment of urinary tract infection is best guided by the result of culture and antimicrobial susceptibility tests. Although the uropathogens causing UTI in pregnancy may be relatively constant, the susceptibility patterns differ in different geographical locations and even within the same country [19]. In our research, the overall susceptibility patterns of isolates to imipenem were 97.3%. Almost all organisms were susceptible to it except one isolate of E. coli and three isolates of Staph aureus. Many researchers have reported high susceptibility of organisms to carbapenems and they remain useful in the treatment of serious infections caused by multidrug-resistant organisms. However, these isolates of E. coli and Staph that were resistant are very significant clinically and should be monitored closely [25]. Susceptibility of uropathogens to nitrofurantoin another potent drug in this study was 76.2%, this was higher than Mokube et al, who reported 29.2%, Onoh et al, 33.3%, Adeyemi et al 56.5% but lower than Okonko et al (84.2%), and Nabbugodi et al, (91.7%)in Kenya [35,36,20, 5, 16]. Overall sensitivity patterns of isolates to Ciprofloxacin were 65.7%, similar results were reported by many researchers but fluoroquinolones are not recommended in pregnancy because they are associated with fetal arthropathy. Nitrofurantoin remains the drug of choice as it is safe to use in pregnancy. The drug is recommended for both asymptomatic and symptomatic urinary tract infection [1]. The low resistance of nitrofurantoin has been attributed to the drug not being used in agriculture [19]. In our study, among E. coli, 100% of isolates were susceptible to nitrofurantoin, this agreed with the work of Igwegbe et al, (100%) in Nigeria, Sevki et al in Turkey (95.4%) but at variance with the work of Onoh et al, (21.1%), Mohemid M. Al-Jebouri (60%), Adeyemi, et al, (61.4%)[37, 38, 36,39,20].

Staphylococcus aureus was 66.7% susceptible to Nitrofurantoin, our result is similar to Iram et al,(66.7%) but higher than Onoh (57.7%) in Abakiliki Nigeria, Adeyemi et al, (51.9%) but lower than Okonko et al, (72.7%)[40, 36,20,5].

Many uropathogens were resistant to most of the antimicrobials used. Overall resistance to most antimicrobial was high 50%-91.7%. cefoxitin (50%), gentamicin (56.3%), amikacin (58.3%), augmentin (66.7%), cotrimoxazole (70.8%), ampicillin (70.8%), cefuroxime (79.2%), ceftazidime (87.5%) and cefotaxime (91.7%). The use of antibiotics that exceeds the resistance rates of 10-20% is associated with an increased risk of therapeutic failure and selection of resistant strains. Therapeutic failure is uncommon in patients with uncomplicated UTI, but the selection of resistant strains may increase the risk of treatment failure which may, in turn, lead to complications because of resistant bacteria [41].

There has been an increasing prevalence of antimicrobial resistance among uropathogens and treatment options are narrowing down. AMR is a serious public health challenge globally [42]. Overuse of antibiotics, inappropriate prescriptions, extensive use of antimicrobial in the livestock sector, availability of few antibiotics, low cost of antibiotics, use of antibiotics without laboratory diagnosis, and lack of regulatory bodies all contributed to the evolution of many drug-resistant microbial strains causing UTI [43, 44, 22, 45].

CONCLUSION:

The prevalence of UTI in this study was 22.9%. Most isolates were sensitive to Imipenem, Nitrofurantoin, and Ciprofloxacin. While most were resistant to conventional antibiotics like augmentin, ampicillin, cotrimoxazole, and gentamicin. Increasing antibiotic resistance to commonly used antibiotics is a challenge to the health-care system. Routine culture and sensitivity must be done before therapy to reduce resistant strains. The government should regulate the use of antibiotics and prohibit over the counter sales and use of antibiotics without a prescription from qualified medical professionals. Regular antibiotic susceptibility surveillance in addition to antibiotic stewardship policy should be adopted.

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