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Antibiotics Susceptibility Study of Uropathogens Isolated from Asymptomatic Pregnant Women Attending a Tertiary Maternity Clinic in Northern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors CA, MU and ADA managed the literature searches and analyses of the study. Authors JAA and AKA performed the statistical analysis. Authors JOO, SPEJ and FSO managed the experimental. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the commonest infections during pregnancy and results in perinatal and maternal morbidity and mortality. For bacterial UTIs, the causative agents have remained virtually the same although with variations in individual prevalence. There has been an increasing resistance by these bacteria to the commonly available antibiotics which has become a serious clinical problem.

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Aim: This work was carried out to determine the prevalence of bacterial uropathogens and their antibiotics susceptibility profile among pregnant women attending a tertiary maternity clinic in northern Nigeria.
Methods: A total of 350 pregnant women aged between 21-55 years were enrolled for this study. Mid-stream, clean catch urine samples were cultured on standard bacteriological culture media. Significant bacteriuria was determined by a presumptive test method. Smears of the urine samples were Gram stained and viewed under the oil-immersion objective, and the numbers of bacterial cells per oil-immersion field per millilitre of urine were noted. The resulting isolates from cultures were morphologically and biochemically characterized based on standard procedures and were further confirmed using Microgen^{TM} GND-ID kits. The antibiotics susceptibility was done using the modified Kirby-Bauer disc agar diffusion method. Statistical package for social sciences (SPSS) version 20 was used for statistical analyses in this study.
Results: Out of 350 urine samples analysed, 83 (23.7%) had significant bacteriuria. The age range of 31-40 years recorded the highest prevalence of 32.5%. Among the isolates, *Staphylococcus aureus* was the most prevalent (29%) followed by *Enterococcus* species (22%) and the least uropathogen was *Streptococcus pyogenes* (3%). Isolated bacteria were resistant to at least two antibiotics with Pefloxacin (55.6%) being highly resisted followed by Ciprofloxacin (50.0%) and Cotrimoxazole (45.6%). Amoxicillin (15.2%), Chloramphenicol (21.1%), Ofloxacin (21.7%), and Amoxicillin –clavulanic acid (29.4%) were more effective against the isolates.
Conclusion: The 23.7% prevalence of bacteriuria and resistance profile of uropathogens to some antibiotics tested in this study is a serious cause for concern. This study showed that uropathogens isolated showed resistance to Pefloxacin (55.6%), Ciprofloxacin (50.0%), and Cotrimoxazole (45.6%) with *Stapylococcus aureus* showing resistance to more number of antibiotics tested. Routine urine culture and antibiotic susceptibility testing is recommended in pregnancy to identify bacterial causes of urinary tract infections for prompt attention and treatment to avoid complications.

Keywords: Antimicrobials; bacteriuria; infectious disease; pregnancy; uropathogens; prevalence.

1. INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. This is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection. It is said to exist when a significant number of microorganisms, usually greater than 10^5 cells per millilitre of urine are detected in properly collected mid-stream "clean catch urine". Among the common infectious diseases, urinary tract infections are commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million. It refers to the presence of microbial pathogens within the urinary tract and is usually classified by the infection site; bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria) [1-3].

In contrast to men, women are more susceptible to urinary tract infection and this is mainly due to their short urethra, absence of prostatic secretion, pregnancy, and easy contamination of the urinary tract with faecal flora [4]. Urinary tract infection in pregnancy is associated with significant morbidity for both mother and baby. The combination of mechanical, hormone, and physiological changes during pregnancy contribute to significant changes in the urinary tract which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy. In addition, as the uterus grows it presses on the bladder and can prevent complete emptying of urine during urination. The stagnant urine is a likely source of infection. Urinary tract infection can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to urinary tract infections are said to have symptomatic bacteriuria. It is a condition characterized by presence of bacteria in two consecutive clear-voided urine specimen both yielding positive culture (> 10^5 cfu/ml) of same uropathogen in patient with classical symptoms, *Escherichia coli* is a major etiologic agent in causing urinary tract infection. Asymptomatic bacteriuria is the occurrence of bacteria in the urine without causing symptoms. The condition may not need treatment. Asymptomatic bacteriuria is only treated in the following circumstances: in pregnant women, in patients who recently had an indwelling catheter removed, before an invasive urologic procedure, in neutropenic patients, or in patients with a

urinary tract obstruction. Chronic prophylactic antibiotic therapy is beneficial in pregnant women with recurrent asymptomatic bacteriuria; if untreated, 20-40% of patients will progress to symptomatic urinary tract infection, including pyelonephritis. Although asymptomatic bacteriuria in adult patients usually does not require treatment, pregnant women should be treated to decrease the risk of pyelonephritis.

Urinary tract infection (UTI) that occurs in a normal genitourinary tract with no prior instrumentation are considered as uncomplicated whereas complicated infection are diagnosed in genitourinary tract that have structural and functional abnormalities, including instrumentation such as indwelling urethral catheter are frequently asymptomatic. Current management of UTIs is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI. There is growing concern regarding antimicrobial resistance worldwide, particularly to *E. coli* which is the dominant causative agent of UTI in pregnant women [5-7].

Urinary tract infection is a common malady of the urinogenital system which may lead to complications in pregnancy, therefore there is need to diagnose and prevent the causative agents from over bearing the urinary tract. This research therefore aims to investigate the prevalence of common uropathogenic agents, and to determine the antibiotic susceptibility profile of uropathogens isolated from pregnant women attending a tertiary maternity clinic in northern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Population

The study was conducted at a University Sickbay in northern Nigeria between the periods of March to September, 2016. The study population comprised of 350 pregnant women of age between 21 and 55 years attending Sickbay Maternity section who gave their consent to be enrolled in the study.

2.2 Collection of Sample

Midstream, clean-catch urine samples were collected into sterile containers from asymptomatic pregnant women attending the University Sickbay. The pregnant women were instructed on how to collect urine sample aseptically. The samples were immediately sent to the laboratory in an ice pack for processing using the standard microbiological methods and examined immediately. All urine samples were analysed within six (6) hours of collection [8].

2.3 Determination of Significant Bacteriuria

A presumptive test method was used to determine significant bacteriuria. Using a sterile Pasteur pipette (one for each sample), one drop of well-mixed, uncentrifuged urine was placed on a slide and Gram-stained. The stained slide was examined under an oil-immersion lens (x600 or more) for presence or absence of bacteria. Samples with one or more bacterial cells per oil-immersion field or more bacteria per millilitre in the specimen were selected as having 10^5 cfu/ml designated as positive [9].

2.4 Bacteriological Procedures

The urine samples were cultured on chocolate agar and MacConkey agar, and samples not cultured within two hours were stored at 4℃. Using a sterile standard wire loop that delivers 0.002 ml of urine, a loopful of urine sample was streaked evenly on dried plates of chocolate agar and MacConkey agar to obtain discrete colonies. The plates were incubated aerobically at 37℃ for 24 hours [10].

2.5 Characterization and Identification of Isolates

Isolates were identified using their colonial morphology, microscopic examinations, and biochemical characteristics based on standard procedures [11,12]. The isolated bacteria were further identified using Microgen^{TM} GNA-ID kit in accordance with the manufacturer's instructions and the Microgen Identification System Software (MID-60) was used for identification of the organisms to species levels.

2.6 Antibiotics Susceptibility Testing

Antibiotic susceptibility of the isolates from asymptomatic bacteriuria to commonly prescribed antibiotics was determined using the modified Kirby-Bauer disc agar diffusion method described by Cheesbrough [13] and CLSI [14]. Antibiotics tested were obtained from Oxoid Ltd and include; Streptomycin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (5 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Amoxicillin–clavulanic acid (30 µg), Gentamicin

(10 µg), Pefloxacin (10 µm), Ofloxacin (30 µg) and Cotrimoxazole (30 µg). A suspension of overnight growth of each isolate on Nutrient agar plates was standardized to 0.5 McFarland scale corresponding to approximately 1.5 x 10^8 cfu/ml. Suspension of the isolates was inoculated on Müeller Hinton agar plate using a sterile swab. The swab was streaked evenly over the surface of the medium to ensure confluent growth. The surface of the agar was allowed to dry for 3-5 minutes, and the antibiotic discs were placed on the surface of the agar using antibiotic disc dispenser. All inoculated plates were inverted and incubated at 35℃ for 16–18 hours. The diameter of each zone of growth inhibition around the discs was measured in millimetre using a pair of compass and a ruler on the underside of the plate. Using the Interpretative Chart [14], the zone sizes of each antimicrobial were interpreted and the organism was reported as 'Resistant', 'Intermediate/Moderately susceptible' or 'Susceptible'. Multidrug resistance (MDR) was defined as resistance to two or more agents in three or more different classes of antibiotics [15].

3. RESULTS

A total of 350 urine samples were cultured for asymptomatic uropathogens out of which 83 (23.7%) were positive for significant bacteriuria (10^5 cfu/ml) among the pregnant women, while 267 (76.3%) of the studied population had no significant bacteriuria (Table 1). Pregnant women within the age bracket of 31-40 years had the highest incidence of positive asymptomatic bacteriuria of 45.8% (Table 2).

Ninety two (92) isolates belonging to six (6) genera (Fig. 1) were recovered from the asymptomatic bacteriuria positive urine samples with mixed infection. The most prevalent organism isolated from asymptomatic subjects in this study was *Staphylococcus aureus* (29%) while the least isolate was *Streptococcus pyogenes* (3%) as shown on Fig. 1. The prevalence of uropathogens increases with increase in age from as low as 10% in age group of 21-30 years to as high as 65% in age group of 41-55 years (Fig. 2).

Table 1. Incidence of asymptomatic bacteriuria among pregnant women

Parameters	Frequency
Number of sample screened	350
Number with significant bacteriuria	83
Number with no significant bacteriuria	267
Number of isolated bacteria	92

The results of our study showed that uropathogens isolated showed resistance to Pefloxacin (55.6%), Ciprofloxacin (50.0%), and

Table 2. Incidence of asymptomatic bacteriuria in relation to age distribution of pregnant women

Age group (yrs)	No of samples	No. negative (%)	No. positive (%)
21-30	129	113 (87.6)	16 (12.4)
31-40	117	79 (67.5)	38 (32.5)
41-55	104	75 (72.1)	29 (27.9)

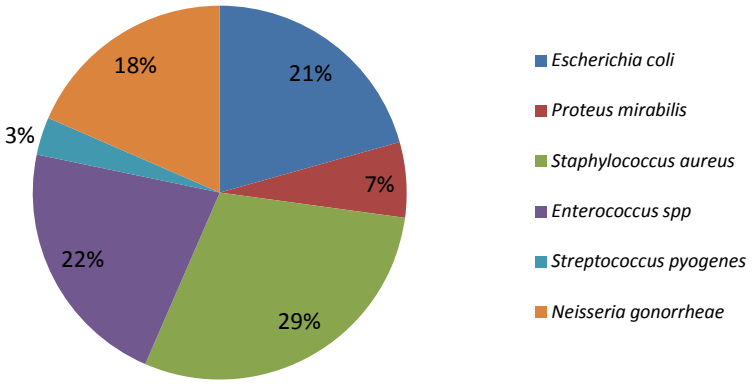

Fig. 1. Percentage distribution of uropathogens

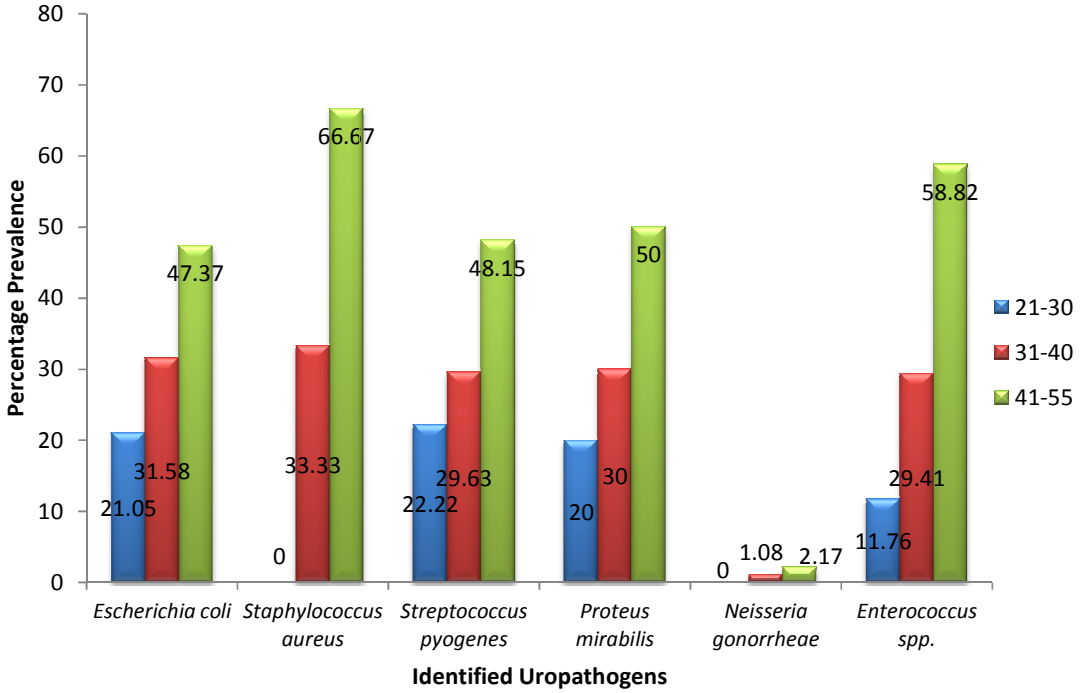

Fig. 2. Age-specific distribution of uropathogens

Cotrimoxazole (45.6%) with *Stapylococcus aureus* showing resistance to more number of antibiotics tested followed by *Streptococcus pyogenes* and *Neisseria gonorrheae*. Amoxicillin (15.2%), Chloramphenicol (21.1%), Ofloxacin (21.7%), and Amoxicillin–clavulanic acid (29.4%) showed a better antibacterial activity against the isolates (Table 3). Our study showed that uropathogens isolated were resistant to two or more antibiotics.

4. DISCUSSION

Asymptomatic bacteriuria is the presence of bacteria in a voided urine sample and is caused by bacterial colonization of the urinary tract. This study found a high prevalence rate of 23.7% among the pregnant women which was comparable to 21.0% reported in Ibadan, Nigeria [16,17]. However, it is lower than 86.6% reported in Benin City, Nigeria [18]. In Enugu, southeast Nigeria, Ezeome et al. [19] reported a prevalence of 15.1% and this is lower than 23.7% found in our study. The high prevalence rate of ASB in pregnancy found in this study indicates the need for routine screening for ASB among the antenatal mothers in our hospital.

The prevalence of 23.7% reported in our study is in disparity with those of MacLean [20] who reported 6% in pregnant women, Hooton et al. [21] who were of the view that asymptomatic bacteriuria affects about 5-10% of both sexually active and pregnant women. This disparity may be related to differences in research locations, community health status, standard of living (socio-economic status), level of health education and availability of healthcare services to prevent recurring cases of bacteriuria in pregnancy. The high prevalence of urinary tract infection reported in this study may be due to pregnancy which reduces the tone of uteri musculature aided by mechanical pressure from the gravid uterus resulting to urinary stasis thus, encouraging bacterial proliferation in urine. Other reasons for the high prevalence rate (27.9 %) within age group 41-55 years could be due to poor genital hygiene practice by pregnant women at this age group who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine. The result of this study is concurrent with those of Nicolle et al. [22] who were of the opinion that for healthy women, the prevalence of bacteriuria increases with advancing age, from ~ 1% among school girls to >20% among healthy women living in the community (Table 2). This corroborated the view of Nicolle et al. [22] that asymptomatic bacteriuria is common, but the prevalence in populations varies widely with age, sex, and the presence of genitourinary abnormalities.

Table 3. Percentage resistance of isolates to antibiotics

Isolates	FREQ.	Antibiotics									
		SXT	CHL	SPX	CPX	AMX	AUG	CN	PEF	OFX	COT
Escherichia coli	21	01(4.8)	(0.00)	02(9.5)	03(14.3)	01(4.8)	0(0.00)	0(0.0)	05(23.8)	03(14.3)	02(9.5)
Streptococcus pyogenes	11	07(63.3)	05(45.5)	01(9.1)	08(72.7)	9(81.8)	5(45.5)	06(54.5)	10(90.9)	9(81.8)	01(9.1)
Enterococcus spp	22	11(50.0)	09(40.9)	07(31.8)	8(36.4)	02(9.1)	05(22.7)	08(36.4)	20(90.9)	04(18.2)	09(40.9)
Proteus mirabilis	07	0(0.0)	0(0.0)	02(28.6)	01(14.3)	01(14.3)	02(28.6)	05(71.4)	0(0.0)	01(14.3)	03(42.9)
Staphylococcus aureus	26	18(69.2)	09(34.6)	16(61.5)	24(92.3)	02(7.7)	14(53.9)	18(69.2)	12(46.2)	0(0.0)	24(92.3)
Neisseria gonorrheae	03	01(33.3)	01(33.3)	01(33.3)	02(66.6)	0(0.0)	01(33.3)	0(0.0)	03(100.0)	3(100.0)	02(66.6)
Total	92	38(41.3)	24(26.1)	29(31.5)	46(50.0)	14(15.2)	27(29.3)	37(40.2)	50(54.3)	20(21.7)	41(44.6)

Key: SXT=Streptomycin, CHL=Chloramphenicol, SPX=Sparfloxacin, CPX=Ciprofloxacin, AMX=Amoxicillin, AUG=Amoxicillin–clavulanic acid, CN=Gentamicin, PEF=Pefloxacin, OFX=Ofloxacin, COT=Cotrimoxazole

The most prevalent bacterium in this study was *Staphylococcus aureus* with the prevalence of 29%. This is in agreement with some researchers whose reports have shown that *Staphylococcus* species is taking the position of *Escherichia coli* as the most prevalent UTI pathogen. Amadi et al. [23] reported 27.1% and 25.4% for *Staphylococcus aureus* and *Escherichia coli* respectively. In a similar study by Ajayi et al., *Staphylococcus aureus* was reported as the commonest cultured organism (72%) and was followed by *Proteus spp.* (14%). Other isolates reported include *Staphylococcus epidermidis* (6%), *Klebsiella spp.* (4%), and *Escherichia coli* (4%) [24]. It was also reported by Akinloye et al. [17] that *Staphylococcus aureus* was the most common (41.3%), followed by *Klebsiella* species (33.3%), and *Escherichia coli* (11.1%) was the least bacteria isolated. However, this observation differs from that of Igwegbe et al. [10], who reported that *Escherichia coli* was the most common isolated organism, (52.6%) followed by *Staphylococcus aureus*, (17.5%) and *Klebsiella sp*., (15.8%). The uropathogens encountered in this study were similar to those recovered in different studies by Morrison and Wenzel [25], Hussain et al. [26], and Park et al. [27]. Adhesion property of the bacteria is an important factor that mediates the ability of a bacterial species to colonise the vaginal or any mucosal surface [28].

Antibiotic resistance observed in this study is a sufficient cause for serious concern. The isolates exhibited resistance to one or more antimicrobials tested. The high resistance profile of uropathogens in this study to Pefloxacin (54.3%), Ciprofloxacin (50.0%), and Cotrimoxazole (44.6%) could have resulted from incomplete course of treatment due to lack of adherence or the use of sub-MIC of the drugs in therapy of previous cases of the disease. This observation is in disparity with those of Ezeh et al. [8] in Zaria, Nigeria, who reported resistance to Quinolones (12%), Cotrimoxazole (17%), and Amynoglycoside (3%). In Benin, Nigeria by Akerele et al. [18] reported that resistance to Ciprofloxacin was 0.3%, and Cotrimoxazole was 21.6%, Okon et al. [29] in Maiduguri, Nigeria reported a similar trend with those of Eze et al. and Akerele et al. who all reported less resistance to the quinolone group of antibiotics. The observed disparity may be as a result of differences in usage and study locations as it is the case with those of Okon et al. and Akerele et al. in which case these bacteria must have acquired resistance to quinolones in the present study due to indiscriminate use of these drugs by subjects in the study region or the organisms must have acquired resistance due to the use of the drugs at their sub-MIC in animal feeds.

5. CONCLUSION

Urinary tract infection is caused by bacterial colonization of the urethra and the most prevalent agent for urinary tract infection found in this study was *Staphylococcus aureus* which causes 29% of the infections. The 23.7% prevalence of bacteriuria and resistance profile of uropathogens to some antibiotics tested in this study is a serious cause for concern. This study showed that uropathogens isolated showed resistance to Pefloxacin (55.6%), Ciprofloxacin (50.0%), and Cotrimoxazole (45.6%) with *Stapylococcus aureus* showing resistance to more number of antibiotics tested. Routine urine culture and antibiotic susceptibility testing is recommended in pregnancy to identify bacterial causes of urinary tract infections and for prompt attention and treatment to avoid complications. The prevalence of urinary tract infections (UTIs) among pregnant women is high, common and increases with age. Highest prevalence is within the age range of 41-55 years.

6. RECOMMENDATION

Good personal hygiene practice should be practiced mostly among pregnant women, drinking of plenty fluids (water) the equivalent of 8 glasses every day to help flush bacteria off the urinary system.

CONSENT

Written informed consent was sought for and obtained from the patients before inclusion in the research.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethical committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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