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# Asymptomatic Bacteriuric Infections and Antibiotic Susceptibility Profile of *Pseudomonas aeruginosa* Isolates in Pregnant Women in Makurdi, Benue State

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**Abstract**

Detection of bacteriuria (ASB) can be reliably done through urine culture. To isolate and characterize bacteria associated with ASB, clean catch, mid-stream early morning urine specimens were collected from 438 pregnant women (age range, 17 to 47 years). Bacterial isolates were morphologically and biochemically characterized using standard bacteriological techniques. Isolates of *P. aeruginosa* were confirmed using API (BioMerieux, USA) rapid tests kits and susceptibility profile of the *P. aeruginosa* isolates were determined by the Kirby-Bauer disc diffusion technique. Two hundred and seventy-seven (63.2%) urine specimens yielded bacteria growth, and the organisms isolated were *Escherichia coli* (87, 31.4%), *Staphylococcus* spp (60, 21.7%), *Proteus* spp (34, 12.3%), *Klebsiella* spp (31, 11.2%), *Pseudomonas* spp (28, 10.1%), *Enterobacter* spp (22, 7.9%), *Streptococcus* spp (10, 3.6%), *Corynebacteria* spp (3, 1.1%) and *Serratia* spp (2, 0.7%). Prevalence of ASB was highest among those in the 21-30 years age group (116, 69.9%), those in their third trimester (126, 70.0%), and those with more than five pregnancies (29, 69.1%), ($\chi^2$ = 0.480; P>0.05). All *P. aeruginosa* isolates were sensitive to the quinolone group of antibiotics - Ofloxacin (OFX) Perfloxacin (PEF) and Ciprofloxacin (CPX) but resistant to Clarithromycin (CLA) (89.3%), Clindamycin (DA) (96.4%), and Ceftriaxone (CRO) (89.3%). The findings in this study have provided epidemiological information and the high prevalence of asymptomatic bacteriuria can be responsible for acute pyelonephritis with its negative outcome such as premature delivery, low birth weight infants, preeclampsia, hypertension, renal failure and foetal death. Approximately 25-30% of asymptomatic bacteriuria in pregnancy is likely to progress to symptomatic infection, therefore asymptomatic bacteriuria should be treated in pregnancy in order to prevent complications.

**Keywords:** Asymptomatic, bacteriuria, pregnancy, Susceptibility, Quinolone

## Introduction

Quantitative analysis of bacteria in urine cultures was developed several decades ago to establish reliable criteria for discriminating between infection and contamination in asymptomatic subjects. Asymptomatic infection might be associated with pyelonephritis, hypertension, renal disease, and complications of pregnancy [1]. Educational programmes that promote the availability of antenatal care and emphasize the importance of an early first antenatal visit (<28 weeks of pregnancy) to the health are paramount to the successful implementation of this intervention. Perhaps most importantly, the availability of adequate diagnostic tools for asymptomatic bacteriuria should be ascertained. In many developing countries, financial constraints may restrict the feasibility of introducing general screening of all pregnant women [2]. Although the incidence of bacteriuria in pregnant women is similar to that in non-pregnant women, the incidence of acute pyelonephritis in pregnant women with bacteriuria is significantly increased. Pregnancy is a unique state with anatomic and physiologic urinary tract changes. While asymptomatic bacteriuria (ASB) in non-pregnant women is generally benign, pregnant women with bacteriuria have an increased susceptibility to pyelonephritis (inflammation of the tissues of the kidney) and low birth weight (birth weight of less than 2500 g) [3,4]; [5], hence special attention to the pregnant women is one of the most important points in health care [6].

The renal pelvis and ureters begin to dilate as early as the eighth week of pregnancy [7] and the bladder itself is displaced anteriorly and anteriorly. Mechanical compression from the enlarging uterus is the principal cause of hydro ureter and hydro nephrosis, but smooth muscle relaxation induced by progesterone may also play a role. Smooth muscle relaxation results in decreased peristalsis of the ureters, increased bladder capacity and urinary stasis. Differences in urine pH and osmolality and pregnancy-induced glycosuria and aminoaciduria may facilitate bacterial growth [8]. Symptomatic urinary tract infections are divided into lower tract (acute cystitis) or upper tract (acute pyelonephritis) infections. Cystitis is defined as significant bacteriuria with associated bladder mucosal invasion, whereas pyelonephritis is defined as significant bacteriuria with associated inflammation of the renal parenchyma, calices and pelvis [9]. Despite universal guidelines recommending screening and treatment of asymptomatic bacteriuria in pregnancy, there is an ongoing debate in the literature regarding the role of asymptomatic bacteriuria in perinatal outcomes. Similarly, controversy exists as to whether antibiotic treated pyelonephritis leads to adverse pregnancy outcomes. Although there have been many articles published on the subject, very little new evidence to address these issues has accumulated since the mid-1970s. Normal urine is sterile: therefore infection could, theoretically, be diagnosed if a single bacterium was isolated from the urinary tract. In practice, voided urine becomes contaminated in the non-sterile distal urethra. Consequently, with logarithmic bacterial proliferation rates, most individuals diagnosed with urinary infection have bacterial counts of $10^4$–$10^5$/ml. Quantitative urine culture is, therefore, a necessity for diagnosis and definition of the concept of any UTI conditions especially when asymptomatic [4][10].

The ability of *Pseudomonas aeruginosa* to cause infection is exacerbated by a high level of resistance to antibiotics, which makes *Pseudomonas* infections difficult to treat [11]. Multi drug resistance *P. aeruginosa* presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome [12]. Unfortunately, selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. Emergence of resistance to multiple drugs due to selection of resistance during anti-pseudomonal therapy among the initially susceptible isolates occurs frequently with this pathogen, as it is more efficient in acquiring drug resistance by various mechanisms.

To determine the prevalence of asymptomatic bacteriuria in pregnant women attending antenatal clinic in Benue State and to evaluate the antibiotic susceptibility profile of these isolates; data on the pathogenesis, natural history, and antibiotic multi drug resistance associated with *Pseudomonas aeruginosa* in asymptomatic urinary tract infection among pregnant women is lacking in Benue State.

## Materials and Methods

### *Location of study*

The study was carried out at the Federal Medical Centre, Makurdi, Middle belt region of Nigeria between September 2014 and April 2015. The hospital is a referral Centre for over half a million people within 40 km radius of the city. The town is divided by the River Benue into the north and south banks. Owing to its location along the banks of the Benue River, Makurdi experiences warm temperature most of the year.

### *Ethical considerations*

The study was approved by the ethics committee of the Federal Medical Centre, Makurdi with an issuance of a letter of introduction and a letter of ethical clearance approval (Ref. N0 FMH/FMC/MED.108/VOL.1/X). Written informed consent was obtained from all patients who participated in the study.

### *Subject selection*

A purposive selection consisting of pregnant women attending the ante-natal clinic was taken. This included women in the three trimesters of pregnancy. Patients were excluded if they had symptoms of urinary tract infection, had taken antibiotics during the previous week, or had any signs of labour. A total of 438 pregnant women attending the ante-natal clinic of the Federal Medical Centre Makurdi participated in this study. Information regarding each patient's medical and obstetric history was extracted from the hospital medical records before collection of urine specimen. Information extracted included the age, parity, gestational age, and occupation.

### *Isolation of bacteria*

The culture media used for isolation were Cystein-Lactose Electrolyte-Deficient, blood and chocolate agar. Each urine specimen was inoculated onto each of the culture medium by streaking with heat-flamed standard wire loop (delivering 0.001 mL urine). The plates were incubated aerobically (37°C, 24h) and then examined. Only specimens yielding significant growth (i.e. containing at least $10^5$ or more organisms per millilitre of a clean-voided midstream specimen per plate) were further analysed [13].

***Identification of isolates***

The morphological characteristics including size, elevation, opacity and colour of the distinct colonies were examined. A representative colony from each significant and homogenous growth was Gram stained as described.Gram-negative rods were identified as lactose or non-lactose fermenters using MacConkey agar, and colonies that did not ferment lactose were presumptively identified as *Pseudomonas aeruginosa* and were confirmed by the oxidase slide and tube agglutination tests. Suspected *P. aeruginosa* containing plates were viewed under UV rays to observe a characteristic fluorescent. Bacterial growth was further sub cultured on selective media using *Pseudomonas* chromagar and centrimide agar, and incubated (37°C, 24 h). Suspected colonies were Gram stained, antibiotic sensitivity and biochemical tests such as citrate, indole, urease, oxidase tests were carried out. Each isolate was identified as *P. aeruginosa* by analytical profile index test kit (API 20 E test; BioMerieux.)

***Antibiotic susceptibility test for P. aeruginosa***

The Kirby Bauer disc diffusion method was employed [14]. Five colonies of each strain of the isolate were inoculated in 5 ml of peptone water (Lab M) and incubated overnight at 37°C. Each overnight broth culture was diluted to cell concentration of $1\times 10^6$ colony-forming units per ml. A sterile cotton-tipped applicator was dipped into the standardized culture ($1\times 10^6$cfu/ml) and was used to inoculate dried plate of sensitivity test agar (STA). The antibiotic discs used were ampicillin (PN) 10 µg, ciprofloxacin (CPX) 10µg, gentamicin (CN) 10 µg. Erythromycin (E) 10µg, nalidixicacid (NA) 30µg, ceftriaxone (CRO) 30µg, Clindamycin (DA) 25µg, clarithromycin (CLA) 10µg, Azithromycin (AZM) 25µg, Perfloxacin (PEF) 1µg, ceporcx (CEP) 5µg, septrin (SXT) 10µg, Augmentin (AU) 30µg, Ofloxacin (OFX) 5µg and Streptomycin (S) 30µg. The plates were incubated at 37°C for 18-24 hours. Zones of inhibitions were measured and interpreted using the chart from CLSI.

***Biochemical tests***

i) **Catalase test**: Using a sterile glass rod, colonies from overnight cultures of the isolates were picked and immersed into a test tube containing 2ml hydrogen peroxide (3% w/v) solution. Immediate production of gas bubbles indicated a positive result [13].

ii) **Citrate test**: Slopes were prepared and streaked with the colonies from overnight cultures of the isolates and the bottom part was stabbed with the inoculating wire, and then incubated at 35°C for 48 hours. The presence of bright blue colouration signified a positive result [13].

iii) **Indole test**: The isolates were cultured in medium (tryptone water) containing tryptophan. Indole production was detected by red colouration which occurred on addition of Kovac's reagent [13].

iv) **Oxidase test**: Few drops of oxidase reagent were placed onto filter paper and colonies of the test isolate were smeared. The phenylalanine present in the reagent would be oxidised to give deep purple colour if the enzyme oxidase is present [13]

v) **Urease test:** Colonies from overnight cultures of the isolates were inoculated into 3 ml of sterile urea broth and incubated at 35 °C for 5 hours. Pink colouration of the medium indicated urease production [13].

***Statistical analysis***

Inferential and descriptive statistics were used for data presentation. Chi-squared test was used to determine association between asymptomatic bacteriuria and socio-demographic characteristics namely: age, parity, trimester, occupation and vaginal wash habit. The significance level was set at $P\leq 0.05$.

## Results and Discussion

***Prevalence of asymptomatic bacteriuria (ASB)***

In the preliminary aspect of the stud, out of four hundred and thirty-eight (438) urine specimens examined, 190 (43.4%) had significant bacteriuria ($\geq 10^5$ cfu/mL) while 86 (19.6%) had non-significant bacteriuria (Table 1).

Most of the bacteria isolated were Gram-negative and the most frequently occurring organisms were *Escherichia coli* (31.6%), *Staphylococcus* species (18.9), and *Pseudomonas aeruginosa* (14.7%). The organisms were *Corynebacterium* species and *Serratia* species, (Table 2)

***Prevalence of asymptomatic bacteriuria in relation to socio-demographic***

The prevalence of asymptomatic bacteriuria (ASB) in relation to age is presented in Table 3. The pregnant women with asymptomatic bacteriuria were aged 17 to 47 years and were from both rural and urban backgrounds. Nearly half of the participants (43.8%) belonged to the 21-30 years age groups while approximately one-twelfth of the participants (8.7%) were below or equal to the 20 years age group. The incidence of significant bacteriuria was highest amongst women in the age group 31-40 years, (50.6%) closely followed by those in the greater than 40 years age group (42.9%). Young women of ages below or equal to 20 years had the least colonization with significant bacteriuria, (23.6%).

**Table 1: Frequency of Bacteriuria Status of Participants**

| Condition | N0./organisms | % |
|---|---|---|
| Significant bacteriuria | 190 | 43.4 |
| Non-significant bacteriuria | 86 | 19.6 |
| No growth/absence of Bacteriuria | 162 | 37.0 |
| Total number of specimens screened | 438 | 100.0 |

**Table 2: Distribution of Bacteria Isolated from Urine of Pregnant Women.**

| Gram reaction | Organisms | Significant Bacteriuria n (%) | Non-Significant Bacteriuria n (%) | Total (%) |
|---|---|---|---|---|
| Gram negative | *Escherichia coli* | 60 (31.6) | 26 (30.2) | 86 (19.6) |
| Gram positive | *Staphylococcus* spp | 36 (18.9) | 24 (27.9) | 60 (13.7) |
| Gram negative | *P. aeruginosa* | 28 (14.7) | 1(1.2) | 29 (6.6) |
| Gram negative | *Proteus* spp | 21(11.1) | 10 (11.6) | 31(7.1) |
| Gram negative | *Klebsiella* spp | 19 (10.0) | 13 (15.1) | 32 (7.3) |
| Gram negative | *Enterobacter* spp | 16 (8.4) | 9 (10.5) | 25 (5.7) |
| Gram positive | *Streptococcus* spp | 7 (3.7) | 1(1.2%) | 8 (1.8) |
| Gram positive | *Corynebacterium*spp | 1(0.5) | 2(2.3%) | 3 (0.7) |
| Gram negative | *Serratia* spp | 2(1.1) | 0(0.0) | 2 (0.5) |
| Absence of bacteriuria | | | | 162 (37.0) |
| | **Total** | | | **438 (100)** |

**Table 3: Prevalence of ASB in Relation to Age**

| Age Group | No. Screened for ASB (%) | Significant ASB (%) | Non-significant ASB (%) | Absence of bacteriuria (%) |
|---|---|---|---|---|
| ≤20 Years | 38(100.0) | 9 (23.6) | 11(29.0) | 18 (47.4) |
| 21-30 yrs | 192(100.0) | 79(41.1) | 41(21.4) | 72(37.5) |
| 31-40 Yrs | 166(100.0) | 84(50.6) | 32(19.3) | 50 (30.1) |
| >40 Years | 42(100.0) | 18(42.8) | 2(4.8) | 22(52.4) |
| **Total** | **438(100.0)** | **190(43.4)** | **86(19.6)** | **162(37.0)** |

**($\chi^2$ = 8.20; df = 3; P = 0.04*);** $\chi^2$ - (Chi-square, df-degree of freedom, *Chi-square is significant at the 0.05 level)

Table 4summarises the prevalence of ASB in relation to parity that is, number of pregnancies reaching viable gestational age. Significant bacteriuria peaked among those who carried more than five pregnancies (59.5%). Incidence of bacteriuria was least among those having their first pregnancies. There was no difference when Chi-square test was used, ($\chi^2$ = 24.72, P = 0.08).

Nearly half of the participants (48.2%) were women in their second trimester that is gestational age between 4-6 months. An increase in significant bacteriuria occurred from the first trimester dropped in the second and peaked in the third trimester implying that with each advancing month of pregnancy, the probability of having bacteriuria increased by 9.6% (p = 0.048) (Table 5).

Table 6presents the prevalence of significant bacteriuria in relation to occupation. Out of 117 house wives, 56 (47.8%) had a high level of bacteriuria even though the highest level occurred among the farmers, 9(56.3%), only one nurse had significant bacteriuria. These differences were however not statistically significant, using the Chi-square analysis, ($\chi^2$ = 4.97, P = 0.42).

The prevalence of significant bacteriuria in relation to vaginal toiletry cleaning habit is shown in Table 7. Nearly 90% that is 374/438 of the respondents affirmed to cleaning the buttock from front to back. A higher level of significant bacteria, (43.9%) occurred among this group over those that cleaned from back to front. There were no statistical differences between method of cleaning the vagina using the Chi-square analysis ($\chi^2$ = 0.91, P = 0.64).

**Table 4: Prevalence of ASB in Relation to Parity (Number of Pregnancy)**

| Parity | Number Tested (%) | Significant ASB (%) | Non-significant ASB (%) | Absence of bacteriuria (%) |
|---|---|---|---|---|
| 1st | 105(100.0) | 41(39.0) | 30(28.6) | 34 (32.4) |
| 2nd | 74(100.0) | 31(41.9) | 15(20.3) | 28(37.8) |
| 3rd | 107(100.0) | 45(42.1) | 16(15.0) | 46(42.9) |
| 4th | 110(100.0) | 48(43.6) | 22(20.0) | 40 (36.4) |
| >5 | 42(100.0) | 25(59.5) | 3(7.2) | 14(33.3) |
| **Total** | **438 (100)** | **190(43.4)** | **86(19.6)** | **162(37.0)** |

($\chi$2 = 24.72; df = 4; P = 0.08); $\chi^2$ - (Chi-square df- degree of freedom)

**Table 5: Prevalence of ASB in relation to Trimester (Age of pregnancy)**

| Trimester (months) | No. Screened (%) | Significant ASB (%) | Non-significant ASB (%) | Absence of bacteriuria (%) |
|---|---|---|---|---|
| 1st (0-3) | 47 (100.0) | 19(40.4) | 8(17.0) | 20(42.6) |
| 2nd (4-6) | 211(100.0) | 78(37.0) | 45(21.3) | 88(41.7) |
| 3rd (7-9) | 180 (100.0) | 93(51.7) | 33(18.3) | 54(30.0) |
| **Total** | **438(100.0)** | **190(43.4)** | **86(19.6)** | **162(37.0)** |

($\chi^2$ = 9.61; df =2; P = 0.048*); $\chi^2$ - (Chi-square, df- degree of freedom*- Chi-square is significant at the 0.05 level)

**Table 6: Prevalence of ASB in Relation to Occupation**

| Occupation | No. Screened | Significant ASB (%) | Non-significant ASB (%) | Absence of bacteriuria (%) |
|---|---|---|---|---|
| Business | 70(100.0) | 28(40.0) | 17(24.3) | 25(35.7) |
| Housewife | 117(100.0) | 56(47.9) | 22(18.8) | 39(33.3) |
| Teacher | 67(100.0) | 28(41.8) | 13(19.4) | 26(38.8) |
| Civil Servant | 110(100.0) | 47(42.7) | 16(14.6) | 47(42.7) |
| Student | 54(100.0) | 21(38.9) | 15(27.8) | 18(33.3) |
| Farmer | 16(100.0) | 9(56.3) | 1(6.2) | 6(37.5) |
| Nurse | 4(100.0) | 1(25.0) | 2(50.0) | 1(25.0) |
| **Total** | **438(100.0)** | **190(43.4)** | **86(19.6)** | **162(37.0)** |

($\chi^2$ = 8.14; df =5; P = 0.62,$\chi^2$ - Chi-square, df- (degree of freedom)

**Table 7: Prevalence of ASB in Relation to Anal Toiletry Cleaning Habit**

| Do you clean from front to back? | No. screened for ASB | Significant ASB (%) | Non-significant ASB (%) | Absence of bacteriuria (%) |
|---|---|---|---|---|
| Yes | 374 (100.0) | 164(43.9) | 75(20.1) | 135(36.0) |
| No | 64(100.0) | 26(40.6) | 11(17.2) | 27(42.2) |
| **Total** | **438(100.0)** | **190(43.4)** | **86(19.6)** | **162(37.0)** |

($\chi^2$ = 0.91; df=2; P = 0.64) $\chi^2$ - Chi-square, df- degree of freedom

***Antibiotic Susceptibility Profile of Pseudomonas aeruginosa Isolates***

Table 8 summarizes the antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates. A total of fifteen different antibiotics grouped into seven classes was used. Most of the isolates (96.4%) were resistant to clindamycin. Resistance was high in both ceftriaxone, and clarithromycin, 25 (89.3%). All Isolates were sensitive to the quinolone group of antibiotics namely, Ofloxacin, Perfloxacin and ciprofloxacin. Also, all *Pseudomonas aeruginosa* isolates were sensitive to both gentamycin and streptomycin.

**Table 8: Antibiotics Susceptibility Pattern of *Pseudomonas aeruginosa* Isolates From Urine**

| Antibiotics | Antibiotic Class | Disc Content (µg) | No.and %Resistant | No. and % susceptible | P –Value |
|---|---|---|---|---|---|
| **Ofloxacin** | Quinolone | 5 | 0(0.00) | 28(100) | < 0.001 |
| **Perfloxacin** | Quinolone | 30 | 0(0.00) | 28(100) | < 0.001 |
| **Ciprofloxacin** | Quinolone | 10 | 0(0.00) | 28(100) | < 0.001 |
| **Augmentin** | Aminoglycoside | 30 | 2(7.1) | 26(92.9) | < 0.001 |
| **Gentamycin** | Aminoglycoside | 10 | 0(0.0) | 28(100) | < 0.001 |
| **Streptomycin** | Aminoglycoside | 30 | 0(0.0) | 28(100) | < 0.001 |
| **Ceporex** | Cephalosporin | 5 | 1(3.6) | 27(96.4) | < 0.001 |
| **Nalidilic Acid** | Nitrofurans | 30 | 3(10.7) | 25(89.3) | < 0.001 |
| **Septrin** | Sulphonamides | 30 | 2(7.1) | 26(92.9) | < 0.001 |
| **Ampicillin** | Penicillin | 10 | 12(42.9) | 16(57.1) | =0.57 |
| **Erythromycin** | Macrolides | 10 | 22(78.6) | 6(21.4) | = 0.005 |
| **Clarithromycin** | Macrolides | 10 | 25(89.3) | 3(10.7) | < 0.001 |
| **Clindamycin** | Macrolides | 25 | 27(96.4) | 1(3.6) | < 0.001 |
| **Azithromycin** | Macrolides | 25 | 6(21.4) | 22(78.6) | = 0.005 |
| **Ceftriaxone** | Cephalosporin | 30 | 25(89.3) | 3(10.7) | < 0.001 |

Numbers in bracket are percentages.

The prevalence of asymptomatic bacteriuria among 438 pregnant women was determined as a pilot or preliminary activity in this research. The results indicated that many (about half) of the urine specimens examined had significant bacteriuria, few of the specimens showed non-significant bacteriuria. Previous studies evaluated the prevalence of asymptomatic bacteriuria and found that it varied from one place to another. In Nigeria, for instance,earlier studies in Benin-City [15] Abakaliki [16] showed a higher prevalence than the results of the present study. On the contrary, prevalence rates lower than what was obtained in this study were reported for Ogun state [17] and Ibadan [40] both in South West Nigeria. In other parts of Africa, particularly Ghana [19] and Ethiopia [20], much lower rates were obtained. Surprisingly similar prevalence rates as obtained in this study were reported in some other parts of the world such as Saudi-Arabia [3], New Zealand [21] and Canada [22]. As far back as the mid–sixties, Sleigh *et al.* [23] obtained low prevalence rates as well. The variation in the prevalence of asymptomatic bacteriuria across the world has been previously reported [2, 21,24].Olusanya [25] and others (Reddy and Campbell, [2, 21,24], for example, suggested that differences in cultural practices among pregnant women across the globe could account for these variations in prevalence rates reported.

Another important finding in this study was the higher frequency of Gram-negative bacteria over the Gram-positive types. According to Tuladhar[26], the presence of a unique structure (for example, the lipopolysaccharide- an endotoxin that elicits a strong immune response when the bacteria infect animals) in Gram-negative bacteria aids attachment of the Gram-negative bacteria to uroepithelial cells, so that the bacteria are protected from urinary lavage thereby allowing bacterial multiplication and tissue invasion that results in invasive infection.

The most frequently occurring bacterium in this study was *E. coli*. This result is consistent with the findings of several studies which found *E. coli* to be the most predominant organism to colonize the urethral meatus and perineum before ascending to the bladder due to its close proximity to the vulva [27, 28, 29]. These factors may explain the relatively high occurrence of *E. coli* among other bacteria isolated from the urine specimens in this work. Nonetheless, contrary to these reports Olusanya[25] reported *Staphylococcus aureus* as the predominant organism isolated from asymptomatic bacteriuria cases.

The result of this study showed that pregnant women within the age group of 31- 40 years had the highest frequency of significant bacteriuria. These results are consistent with earlier studies [19] which concluded that since women in the age group 25-40 years are more sexually active, acquiring asymptomatic bacteriuria was probably a risk factor. In general, therefore, it is likely that most of the anatomic and physiologic changes in pregnancy influence the urinary tract and can result in urologic diseases and changes in kidney function. These conditions are serious threats for both the mother and child and may further result in hypertension, preeclampsia, low birth weight, prematurity, septicaemia, maternal and foetal death [30]; Christensen[31]. High incidence of urinary tract infection has been attributed to hormonal effects observed in pregnancy which reduces tone of the ureteric musculature coupled with mechanical pressure from the gravid uterus leading to urinary stasis, hence encouraging bacterial proliferation in urine [24]. Screening in pregnancy for asymptomatic bacteriuria, and treatment with antibiotics of positive cases has been recommended in order to prevent complications such as pyelonephritis, premature labour, still birth, hypertension, maternal anaemia, preeclampsia and septicaemia [32, 33]. The result of chi-square test obtained in this study showed that age is significantly associated with asymptomatic bacteriuria and implies that age has a role to play in acquisition of significant bacteriuria.

In the current study, women who have had more than five pregnancies had the highest frequency of significant bacteriuria, even though chi-square test showed that there was no statistically significant association between bacteriuria and parity. This is in line with reports that the prevalence of bacteriuria increases with age, sexual activity and parity. On the contrary, Reddy and Campbell [21] had observed a higher incidence of bacteriuria among under 21 year old primigravid- women pregnant for the first time and a lower incidence in multiparous women over 35 years old.

A clinically relevant finding in this work is the statistically significant association between bacteriuria and age of pregnancy (trimester). Significant bacteriuria was highest among women in the gestational age of 6-9 months (third trimester). This result agrees with the finding of Nicolle [34] that the risk of acquiring bacteriuria increases with gestational age. Likewise, Lindsay [35] attributed the high incidence of bacteriuria to –increased gestational age: hormonal activity is increased at the period of pregnancy and aids the growth of bacteria. Conversely, Nnatu [36] reported highest incidence of bacteriuria in the second trimester of pregnancy and a lower incidence in the first and third trimester.

Significant bacteriuria was found to be higher in pregnant women who were farmers and housewives. The level of education attained by a person contributes to bacteriuria among pregnant women, according to Nseobong [37]. Series of hospital antenatal patients have been studied and varying relationships between social classes and bacteriuria have been reported earlier in literature. Nicolle [34] reported that the prevalence of ASB in pregnant women was higher among individuals in lower socio economic classes. In our country the effect of socio-economic status that is education, occupation, income and family structure seem to be consistently low among housewives. In general, therefore, it seems that these conditions are less likely to occur in other occupation since most well have better background for hygienic practices.

In this study, manner of cleaning the anus after toilet activity was not significantly associated with bacteriuria, even though women who cleaned their anus from front to back had higher rate of significant bacteriuria. This finding is contrary to previous studies which suggested that cleaning buttocks or anus from back to front (a bad practice) may dislodge uropathogens from rectal flora to enter the urinary tract via the urethra into the bladder. However, the findings of the current study do not support these previous findings as women who washed from back to front had a lower frequency of bacteriuria. It could be argued that the positive results may be attributed to a combination of mechanical, hormonal and physiologic changes that occur during pregnancy. These changes may cause alteration in the urinary tract, and may have profound impact on the acquisition of bacteriuria during pregnancy. Such as dilatation of the ureter, decrease in ureteral peristalsis, and decrease in bladder tone. As suggested by Davis and Flood [38], the physiologic increase in plasma volume during pregnancy decreases urine concentration and increases urinary progestins and oestrogens leading to a decreased ability of the lower urinary tract to resist invading bacteria. This factor may account for the high frequency of significant bacteriuria in pregnancy.

Again, host genetic factors among pregnant women may influence susceptibility to ASB [38]. Susceptible patients may have a genetically increased number of receptors on uroepithelial cells to which bacteria may adhere. Poor genital hygiene practiced by antenatal women [39] may also contribute to high incidence of ASB. Pregnancy may make it difficult for the pregnant women to clean their anus and genitals properly after defecating or passing urine due to their protruded bellies; they usually find it difficult to bend and squat. Probably other factors like mechanical, physiological, hormonal and even genetic features could account to this rather unexpected finding.

The presence of *Pseudomonas aeruginosa* in the urinecan cause septic arthritis (painful infection in the joint) in pregnant subjects [40]. The high occurrence of *P. aeruginosa* in the pregnant women corroborates the findings of Mittal and Wing [41] that with increase in age and pregnancy, immunity is impaired. Incidence of resistance to either a single chemotherapeutic agent or many agents at the same time (multiple-drug resistance) have been reported among many pathogenic organisms [42, 43, 44, 45, 46]. Antimicrobial resistance encountered in clinical practice could be inherent or genetically acquired [47].

The choice of antibiotic should however be based on urine culture, stage of gestation, clinical data and the characteristics of the antibiotic [48]. Although aggressive antibiotic treatment may be necessary to reduce the risk of pyelonephritis and other complications of asymptomatic bacteriuria in pregnancy [45], this should be done with caution as it is known that with the indiscriminate use of these antibiotics, urinary pathogens are becoming multidrug resistant [49]. A number of other fluoroquinolones including ofloxacin, enoxacin, lomefloxacin, temafloxacin, fleroxacin have now [1]been formulated and used clinically. Quinolones possess excellent in vivo activity against *Enterobacteriaceae* including *P. aeruginosa* and other genera in this Family of bacteria [50].

From this study *P. aeruginosa* exhibited high level of resistance to erythromycin, clarithromycin, clindamycin and ceftriaxone. Most of the isolates had Multiple Antibiotic Resistance (MAR) index ≥0.2 which is taken as significant, revealing that they were resistant to more than two classes of antimicrobial agents tested. This observation suggests that the isolates in this study may probably have originated from an environment where antibiotics are often used indiscriminately [51]. Broad-spectrum antibiotics are sometimes given in place of narrow-spectrum antibiotics as a substitute for culture and sensitivity testing, with the consequent risk of selection of antibiotic-resistant mutants [52, 53, 54].

There was resistance to beta-lactam drugs in this study. This result is consistent with those of Olusanya *et al.* [25] more than half of the bacteria isolated produced beta-lactamase. This enzyme hydrolyses the beta-lactam drugs such as the penicillins and cephalosporins, though the cephalosporins are reported to be more stable. All *Pseudomonas aeruginosa* isolates were sensitive to ofloxacin, perfloxacin and ciprofloxacin (the quinolones) and to gentamicin. These results differ from those of Olusanya[25]resistance to beta-Lactam drugs such as Penicillin may also be co transferred with resistance to other antibiotics such as gentamicin, tetracycline and even chloramphenicol. A similar finding has been reported among bacterial from cases of acute otitis media in Ile-Ife, Southwestern Nigeria [55].

*Pseudomonas aeruginosa* had the highest resistance to Erythromycin, Clarithromycin and Clindamycin (the Macrolides). The yield in this investigation were higher compared to the study of Kirst and Sides [56]. Screening for young teenage parous women and those coming from disadvantaged socio-economic conditions and those with a past history of urinary tract infection is recommended before antibiotics is administered [3].Olusanya[25], on the other hand, advocated routine screening for all pregnant women at least during the first visit to the ante-natal clinic.

## Conclusion

The presence of at least $10^5$ colony forming units per mL of urine, of a single uropathogen, and in a midstream clean-catch specimen is considered a positive test result. Our results which show that about four in every ten pregnant women have significant bacteriuria support routine screening of pregnant women for bacteriuria as part of antenatal care. Untreated pregnant women with significant bacteriuria are known to have higher complications in pregnancy than those treated.

**Declaration of conflicting interests**

The authors declared no potential conflicts of interest

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