Bacteriuria of women of child bearing age

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ABSTRACT

Urinary tract infection (UTI) is one of the most common diseases encountered worldwide. In this study, urine samples were collected from thirty women of child bearing age (18-25 years) to determine the prevalence of bacteriuria, as well as the presence of pus cells and white blood cells which are signs of infection. The urine microscopy result revealed that 7 (23.3%) of the sample had greater than 10 leucocytes per high power field. Only four of the samples had a significant number of pus cells. The bacteria count recorded ranged from 25 cfu/0.001 mL to 276 cfu/0.001 mL. Of the samples, 13 were positive for bacteriuria, having bacterial count greater than 100 cfu/0.001 mL. The bacteria isolates include *Klebsiella* spp. (3.8%), *Streptococcus* spp. (2.5%), *Escherichia coli* (62.5%) and *Staphylococcus* spp. (21.2%). Of these, *Klebsiella* spp. was the least prevalent and *Escherichia coli* had the highest number of occurrence. These organisms were very sensitive to Ciprofloxacin, Pefloxacin, Zinnacef, and Gentamycin according to the antimicrobial susceptibility test carried out. Ciprofloxacin was found to be the most effective antibiotics against all the bacterial isolates. Here, zones of inhibition ranged from 15 mm (*Streptococcus* spp.) to 20 mm (*Escherichia coli*). All the bacterial isolates were resistant to Amoxicillin, with zones of inhibition between 11 (*Klebsiella* spp., *Escherichia coli, Staphylococcus* spp.) and 13 mm (*Streptococcus* spp.). Since the prevalence of asymptomatic bacteriuria is high, early diagnosis and proper treatment is necessary in order to prevent it from becoming symptomatic.

Keywords: Asymptomatic bacteriuria, Urinary tract infection, Prevalence, Bacterial isolates, Antibiotics susceptibility

1. INTRODUCTION

Infections of the urinary tract (UTI) are one of the most common infections for which antibiotics are prescribed. Asymptomatic bacteriuria or asymptomatic urinary tract infection is a common condition (Asscher, 2000). Bacteriuria is considered to be a common and important

complication of pregnancy (Patterson *et al.*, 2003). Based on the microbiological definition, urinary tract infections are characterized by the presence of $\geq 10^5$ colony forming units per mL (cfu/mL) of a single bacterial species or multiple organisms in two consecutive urine specimen, properly collected from a person with symptoms or signs of UTI (Rubin *et al.*, 2006). However, the presence of a significant quantity of bacteria in the urine of an asymptomatic patient is known as asymptomatic bacteriuria. Asymptomatic bacteriuria is present when there are persistent actively multiplying bacteria within the urinary tract without symptoms or without clinical features of UTI (Cunningham *et al.*, 2001; Miller *et al.*, 1997; Smaill, 2006). Quantitative criteria to establish the diagnosis of significant bacteriuria in an asymptomatic person are: at least 10^5 cfu/mL of urine in a voided midstream, so called clean-catch specimen and at least 100 cfu/mL of urine obtained by bladder catheterization. Despite several decades of research, there is still a considerable amount of controversy about the adequate management of bacteriuria.

A common dilemma in clinical medicine is whether or not to treat asymptomatic bacteriuria (Colgan *et al.*, 2006). It is now recognized that certain patient characteristics can lead to the development of symptomatic infections based on an asymptomatic bacteriuria (Garigalo, 2000). For several clinical scenarios, the antibiotic treatment of asymptomatic bacteriuria has been shown to improve patient outcomes. Based on the clinical trials conducted over the last few decades, better recommendations for the management of bacteriuria in different patient population could be established. The infectious diseases society of America (IDSA) established guidelines for the screening and treatment of asymptomatic bacteriuria (Nicolle *et al.*, 2005).

The optimal management depends significantly on specific patient characteristics, co-morbidities, and risk factors. Recent studies could prove that for certain patient populations, screening and treatment are beneficial or may be beneficial. However, for other clinical scenarios, screening and treatment for asymptomatic bacteriuria has not proven to be beneficial, therefore is highly controversial. Importantly, over treatment of asymptomatic bacteriuria is a quality, safety and cost issue (Nicolle *et al.*, 2005). Asymptomatic bacteriuria is common, with varying prevalence by age, sex, sexual activity, and the presence of genitourinary abnormality (Nicolle, 2003; Zhanel *et al.*, 2007).

In healthy women, the prevalence of bacteriuria increases with age, from about 1% in females 5-14 years of age to more than 20% in women of at least 80 years of age living in the community (Nicolle 2003). Several factors are postulated to be associated with an increased asymptomatic bacteriuria (ASB). Structural urinary tract abnormalities, such as the presence of renal calculi are thought to predispose to bacteriuria by causing irritation and inflammation of the urinary tract mucosa, restricting urinary flow which leads to stasis and obstruction (Nicolle 2003). There has been a considerable controversy about the appropriate management of bacteriuria. Evidence reported in clinical trials, undertaken over the past three decades, however, is sufficient to support recommendations for management in most populations.

1. 1. Aim and Objectives

(1) To determine the prevalence of bacteriuria among women of child bearing age.
(2) To isolate and identify the associated bacteria in women of child bearing age.
(3) To determine the prevalence of each bacterium in the urine samples collected.
(4) To determine the antibiotic susceptibility pattern of the isolated bacteria.

2. EXPERIMENTAL / RESULTS

2. 1. Sample Collection

30 urine samples (mid-stream urine) comprising 5 each were aseptically collected using sterile containers from women of child bearing age from 6 different hostels in Abraka, Delta State, Nigeria, namely: Council hall, Ethiope hall, Olessoh hall, Americana hostel, Our Saviour meridian, and King David hostel. The women were educated on how to collect the urine in order to avoid contamination before the samples were taken, and the urine samples were immediately transported to Delta State University Microbiology Laboratory for analysis.

2. 2. Sterilization of materials and working surfaces.

All working surfaces were swabbed with disinfectant; culture media were sterilized with an autoclave at 121 °C for 15 minutes at 15psi while wire loop was sterilized by flaming.

2. 3. Microbiological analysis of urine sample

2. 3. 1. Macroscopic examination

The colour or appearance of each urine sample was observed and recorded.

2. 3. 2 Microscopic examination

The different urine sample was centrifuged at 15000 rev/min for 5 minutes and then the supernatants were discarded by inverting the test tubes. A wet prep was then made on a grease free slide for each urine sample and examined under the microscope for the presence of pus cells and white blood cells using 40× objectives.

2. 3. 3. Urine culture

Each urine sample was gently shaken and then inoculated on CLED, MacConkey and nutrient agar using a 0.001 mL wire loop for 24 hours after incubation, colony counts were made and their morphological characteristics were also recorded and were performed and interpreted according to Kass criteria (1957) which state that the presence of >100 cfu/0.001 mL is indicative of bacteriuria.

2. 3. 4. Obtaining of Pure Culture

Different characteristics colonies were sub-cultured into different nutrient agar plates and then incubated at 37 °C for 24 hours.

2. 4. Characterization and Identification of Isolates

All isolates were identified by standard methods, as described by Cowen and Steel (1994) which include:

2. 4. 1. Gram Staining

Smear of the different isolates were made on clean slide using a sterile wire loop and normal saline, air-dried and then heat fixed by passing the slide over flame for 2-3 times . The slides were then flooded with crystal violet and allowed to stand for 30 seconds before they

were washed off with water. Lugol's iodine was then added to stained smear, allowed to stand for another 30 seconds and then washed off with water. Alcohol was then used to decolourize the smear which was then washed off immediately. The smears were then counterstained with safranin and allowed to stand for 1 minute before washing off the stain with water. The slides were then air-dried and viewed under the microscope using 100× objectives after the addition of a drop of oil immersion. Gram positive organisms appear purple, while gram negative organisms appear pink.

2. 4. 2. Catalase Test

A loopful of a 24-hour culture was placed on a clean slide after which 1-2 drops of hydrogen peroxide was then added. Evolution of gas bubbles indicates a positive test while the absence of gas bubbles indicates a negative test.

2. 4. 3. Motility Test

A nutrient agar slant was prepared after which it was stabbed with the test isolate and incubated at 37 °C for 24 hours. Growth of the organism/isolate along the line of stab indicates a negative result, while growth away from the line of stab indicates a positive result.

2. 4. 4. Indole Test

3 mL of peptone water was inoculated with the test isolate and then incubated at 37 °C for 24 hours. 0.5 mL of Kovac's reagent was then added to the culture and then gently shaken and observed after 1 minute. The development of a reddish brown layer at the surface of the culture indicates a positive result.

2. 4. 5. Citrate Utilization Test

The test isolate was inoculated into Simmon citrate agar slant and incubated for 24-48 hours at 37 °C. A change in colour of the medium from green to blue indicates a positive result.

2. 4. 6. Oxidase Test

Few drops of tetramethyl-p-phenylenediamine dihyrochloride were placed on a piece of filter paper. The test isolate was then smeared across the surface of the filter paper. Development of a dark purple colour within 10 seconds indicates a positive test.

2. 4. 7. Triple Sugar Iron (TSI) test

TSI agar slant was prepared in a McCartney bottle and then inoculated with the test organism by stabbing and streaking, respectively, followed by incubation at 37 °C for 24-28 hours. The possible results are:

- A yellow bottom (acid production) and red pink slope indicates the fermentation of glucose only.
- Cracks and bubbles in the medium indicate gas production from glucose fermentation.
- A yellow slope and yellow bottom indicates the fermentation of lactose and glucose.
- A red-pink slope indicates no fermentation of glucose or lactose.

- Blackening along the line of stab or throughout the medium indicates hydrogen sulphide (H$_2$S) production.

2. 5. Sensitivity Test

The different bacterial isolates were heavily inoculated into freshly prepared nutrient agar plates and then antibiotic doses were then placed in each plate aseptically using forceps. The plates were then incubated for 24-48 hours at 37 °C and after incubation, the zones of inhibition produced were measured and recorded.

Table 1. Microscopic Examination and Physical appearance of Urine Samples.

Sample	Physical appearance of urine	Wet prep (microscopy)
1	Pale yellow and clear	14 WBCs and scanty pus cell
2	Amber and clear	1 WBC
3	Amber and clear	1 WBC
4	Pale yellow and clear	1 WBC
5	Yellow and clear	Many pus cells
6	Yellow and clear	5 WBCs
7	Amber and clear	3 WBCs
8	Pale yellow and clear	2 WBCs and scanty pus cell
9	Amber and clear	7 WBCs and few pus cell
10	Amber and clear	1 WBC
11	Amber and clear	3 WBCs and few pus cell
12	Amber and clear	4 WBCs and few pus cell
13	Yellow and clear	4 WBCs and few pus cell
14	Amber and clear	7 WBCs
15	Pale yellow and clear	4 WBCs
16	Yellow and clear	11 WBCs
17	Amber and cloudy	14 WBCs
18	Amber and clear	3 WBCs
19	Amber and clear	25 WBCs and many pus cell
20	Yellow and cloudy	15 WBCs and many pus cell
21	Pale yellow and clear	1 WBC
22	Amber and cloudy	18 WBCs and many pus cell
23	Amber and clear	4 WBCs, few pus cell
24	Yellow and clear	4 WBCs
25	Pale yellow and clear	7 WBCs, few pus cell
26	Pale yellow and clear	3 WBCs
27	Pale yellow and clear	1 WBC
28	Amber and clear	5 WBCs, few pus cell
29	Yellow and clear	9 WBCs, few pus cell
30	Pale yellow and clear	3 WBCs, few pus cell

WBC – White Blood Cell

The microscopic examination of the wet prep indicated the presence of pus cells and white blood cell. 10 of the urine samples collected were pale yellow in colour, 14 were amber in colour, while 6 had yellow colour, as shown in **Table 1**. It also shows that pus cells were present in most of the urine samples, although they were few in number, while some urine samples had more than 10 WBCs.

Table 2 indicates the presence of bacteriuria in 13 (43.3%) of the urine samples, while the bacteria count of the remaining 17 (56.7%) urine samples were insignificant, since they were less than 100 cfu/0.001 mL, according to Kass Criteria (2000).

Table 2: Bacterial count of the different urine samples.

Sample count (cfu/mL)	Bacterial
1	25
2	54
3	66
4	61
5	55
6	148
7	53
8	168
9	71
10	102
11	55
12	65
13	134
14	45
15	76
16	132
17	276
18	87
19	139
20	240
21	83
22	112
23	75
24	163
25	201
26	137
27	84
28	122
29	96
30	47

Table 3. Morphological/Biochemical characteristics of bacterial isolates.

	Isolates			
	Staphylococcus spp.	*Escherichia coli*	*Klebsiella* spp.	*Streptococcus* spp.
Cultural Characteristics	Creamy, convex smooth edge	Pink, convex colonies with smooth edges	Creamy, smooth edges and convex colonies	Creamy and raised colonies
Morphological Characteristics	Cocci in clusters	Rods	Rods	Cocci in chains
Gram stain	+	-	-	+
Catalase	+	+	+	-
Oxidase	-	-	-	-
Citrate	-	-	+	+
Indole	+	+	-	-
H_2S	-	-	-	-
Motility	+	+	-	+
Glucose	A/G	A/G	A/G	-
Lactose	-	A/G	A/G	-

+ = positive, − = negative,   A/G = Acid and Gas production
A = Acid production only,   G = Gas production only

Table 4. Prevalence rate of different bacteria isolates in the urine samples collected.

Bacteria isolate	Number of occurrence	Percentage (%)
Klebsiella spp.	4	3.8
Streptococcus spp.	13	12.5
Escherichia coli	65	62.5
Staphylococcus spp.	22	21.2

As shown in the **Tables 3** and **4** above, *Escherichia coli* has the highest number of occurrence, while *Klebsiella* spp. was the least prevalent bacterium.

Table 5. Antibiotics Susceptibility pattern of the different bacteria isolates.

Isolates	Antibiotics (zone of inhibition in mm)													
	S	OFX	SXT	CH	SP	CPX	AM	AU	CN	PEF	LEV	Z	APX	E
Klebsiella spp.	8	10	11	12	10	18	11	10	16	16	-	-	-	-
Escherichia coli	12	11	10	13	12	20	11	9	15	14	-	-	-	-
Staphylococcus spp.	16	-	-	13	-	15	11	-	12	13	11	15	11	14
Streptococcus spp.	12	-	-	15	-	16	14	-	13	15	16	16	14	16

S - Streptomycin   OFX – Tarivid   SXT – Septrin   CH – Chloramphenicol
SP – Spavfloxacin   CPX – Ciprofloxacin   AM – Amoxicillin   AU – Augmentin
CN – Gentamycin   PEF – Pefloxacin   LEV – Levofloxacin   Z – Zinnacef
APX – Ampiclox   E - Erythromycin

Ciprofloxacin (CPX), Gentamycin (CN), Pefloxacin (PEP) were the most effective antibiotics against the bacterial isolates (**Table 5**). *Streptococcus* spp. was strongly inhibited by almost all the drugs used.
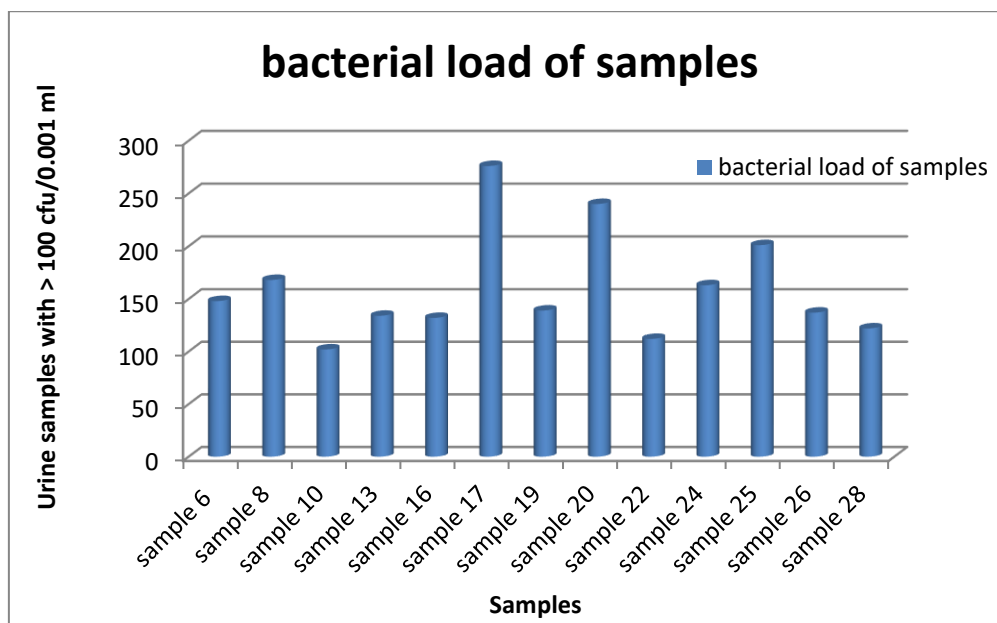

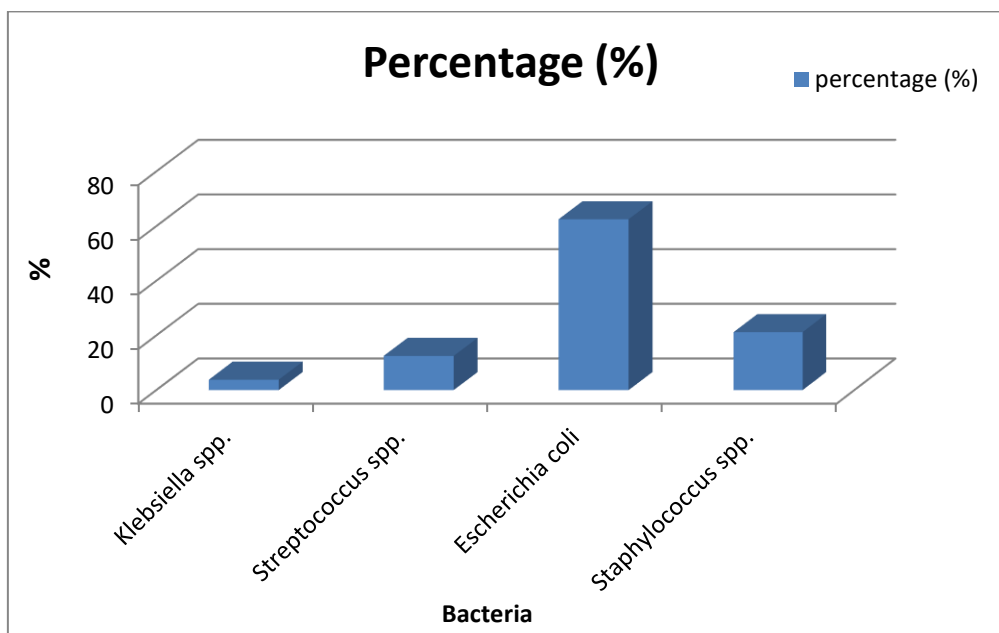**Figure 1.** Bacterial load of urine samples with > 100 cfu/0.001 mL

Figure 2. Prevalence rate of different bacteria isolates in the urine samples

3. DISCUSSION

In the study carried out, 30 urine samples were collected from 30 different women of child bearing age to determine the prevalence of asymptomatic bacteriuria as well as the prevalence of pus cells and white blood cells which are signs of infection (**Figure 1**).

The urine samples that were pale yellow in colour form 33.3% of the total sample, 20% had yellow colour, while 46.7% were amber in colour, as shown in Table 1. This could be attributed to the nature or type of food taken by the individuals from which the urine sample were collected, which agrees with the report of Nicolle (2003) who stated that the type of diet taken by a person could affect the colour of urine. Also 23 (76.7%) of the samples had less than (<) 10 white blood cell per high power field, and 16 (53.3%) contained no pus cells. These results were interpreted based on Kass criteria (1957).

Using this same criteria for bacteria growth in urine (100 cfu/mL using 0.001 mL wire loop), 13 (43.3%) of the samples collected had >100 cfu/mL which is indicative of bacteriuria while 17 (56.7%) had negative result, as shown in Table 2. These results could be attributed to the level of personal hygiene practiced by these women. Sexual intercourse is the most important risk factor for bacteriuria in women of child bearing age. The use of spermicidal jelly is thought to further increase the risk of developing a UTI by altering the virginal flora in favour of colonization with urinary pathogens. This is consistent with the findings of Chaudhry *et al*., (2002), where 20% of the samples collected from adult females were negative for bacteriuria.

Of all the organisms isolated, *Klebsiella spp.* was the least prevalent (3.8%), *Streptococcus* spp. (12.5%), *Staphylococcus* spp. (21.2%), while *E. coli* had the highest number of occurrence (62.5%), as shown on Table 4. *E. coli* is a known causal organism of urinary tract infection worldwide and this account for its high prevalence in the samples collected (**Figure 2**). *Klebsiella* spp., *Staphylococcus* spp. and *Streptococcus* spp. have all been implicated in

asymptomatic bacteriuria by several studies (Nicolle, 2003, 2005, 2006). This is also in agreement with the work of Chaudhry *et al*., (2002) in which these organisms were isolated from urine samples.

Table 5 shows the antibiotic susceptibility test in which *Streptococcus* spp. was sensitive to almost all the antibiotics used, of which Levofloxacin, Zinnacef and Erythromycin were the most effective with zones of inhibition of 16 mm each. This could be attributed to the nature of the cell wall of *Streptococcus* spp. Levofloxacin, Ampiclox, and Amoxicillin were the least effective antibiotics against *Staphylococcus* spp. as they all produced a zone of inhibition of 11 mm each. On the other hand, the gram negative bacteria *Klebsiella* spp. and *E. coli* were also inhibited by Ciprofloxacin, Gentamycin, and Pefloxacin with zones of inhibition ranging from 15 mm to 20 mm, with Ciprofloxacin having the most inhibitory effect.

The strong inhibitory effect of Ciprofloxacin may probably be due to its less use since it is comparatively a newer introduction and also costly. *Klebsiella* spp. and *E. coli* were resistant to Streptomycin, Tarivid, Septrin, Chloramphenicol, Spavfloxacin, Amoxicillin, and Augumentin with zones of inhibition between 8 mm and 12 mm. The gram negative bacterial isolates were more resistant to the antibiotics used as against the gram positive bacterial isolates which were susceptible to the antibiotics used.

This agrees with Nicolle (2005) who reported that gram negative bacteria are more resistant to antibiotics than gram positive bacteria due to the presence of their cell wall's lipopolysaccharide. According to Colgan *et al*., (2006), there are few scenarios in which antibiotic treatment of asymptomatic bacteriuria has been shown to improve patient outcomes, but because of increasing antimicrobial resistance, it is important not to treat patients with asymptomatic bacteriuria unless there is evidence of potential benefits.

4. CONCLUSION

This study indicates the prevalence of asymptomatic bacteriuria among women of child-bearing age has increased over the years and this could be attributed to several factors, such as poor personal hygiene, low standard of living, as well as the increasing rate of sexual indulgence common among young women. Wherever urine culture indicates positive result, a proper and immediate treatment should be initiated since there is a tendency for the asymptomatic bacteriuria to become symptomatic particularly during pregnancy.

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