



Bacteria and Urinalysis Profile of Asymptomatic Bacteriuria in Women in Lagos, Nigeria

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Abstract

Urine epidemiological surveillance from outpatient clinic patients can provide insights into urinary pathogen prevalence and antibiotic susceptibility. The goal of this study was to find the most common bacterial isolates associated with ASB in women in Lagos, Nigeria. The study also evaluated the antimicrobial susceptibility pattern of the isolated bacteria. Urine samples were collected from women attending the outpatient clinic of the Lagos University Teaching Hospital in Lagos State, Nigeria, who were 18 years or older, did not have symptoms of urinary tract infections, and were not currently on antibiotics. The samples were tested for significant bacteriuria using microscopic methods, and urine culture and urinalysis were performed using a commercial test strip. Antimicrobial susceptibility testing was performed to evaluate the isolated bacteria's antibiotic susceptibility profile. Of the 162 women included in the study, 45 had significant bacteriuria. These women, however, yielded 48 bacterial isolates. The most common urinary pathogen isolated was *Staphylococcus aureus*, followed by *E. coli*. All of the bacteria were susceptible to imipenem and nitrofurantoin, but there was significant resistance to ampicillin, augmentin, and tetracycline. The presence of nitrites in the urine was found to be significantly associated with significant bacteriuria, while the presence of leucocytes in the urine was found to be associated with staphylococcal infection. Staphylococci species appear to be the new most common cause of ASB and, by extension, urinary tract infection. Nitrofurantoin and vancomycin are effective treatments for ASB. The use of urinalysis results to establish significant bacteriuria is insufficient.

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Introduction

Apart from the distal urethra, the genitourinary tract, normally, is usually sterile. Asymptomatic bacteria, however, occurs when bacteria ascends from the urethra into the bladder, and sometimes into the kidneys [1]. Asymptomatic Bacteriuria (ASB) is the presence of bacteria in the urine without the presence of Urinary Tract Infection (UTI) symptoms. In most cases, the bacteria that typically colonize the urinary tract do not cause diseases as the host has several methods for removing bacteria from the system, some of which include the host's innate and adaptive immune response and urination [2,3]. Bacteria that cause UTI either have characteristics that allow them to survive in the urinary tract, such as biofilm formation, adhesins, urothelial cell invasion, toxins, and siderophores, or are present in catheterized patients who are unable to remove bacteria, or are introduced into the genitourinary tract without prior host colonization [4-8]. However, bacteria that cause ASB persist in the urinary tract without provoking an immune response from the host sufficient for the production of symptoms or for the eradication of the bacteria. Among the factors that can influence the persistence of bacteria in the urine are the genetic predisposition of the host, such as obstructive uropathy, incomplete bladder emptying, fecal soiling of the perineum, the presence of an indwelling urinary catheter, and frequent instrumentation of the urinary tract [8-12].

The most commonly isolated organism associated with ASB is *Escherichia coli* (*E. coli*), followed by other *Enterobacteriaceae* such as *Klebsiella* species, *Citrobacter* species, *Proteus* species, and *Providencia* species, and then followed by other gram negative bacteria such as *Pseudomonas aeruginosa*. *Enterococcus* species, *Streptococcus* species, *Staphylococcus aureus*, coagulase negative *Staphylococcus aureus*, and other gram positive bacteria are also isolated from the urine of cases with ASB [1,11,13]. Most studies have often reported *E. coli* as the most commonly isolated bacteria

in ASB. A study that conducted a systematic review of the literature on antimicrobial resistance in bacterial isolates from ASB in pregnant women found *E. coli*, *Klebsiella* sp., *P. aeruginosa*, *Staphylococcus aureus*, *Proteus* sp., and *Enterobacter aerogenes* agents were the most frequently isolated pathogens [14]. Similarly, a systematic review in Africa also found *E. coli* as the most common bacterial isolate in ASB, with a pooled prevalence of 33.4%. However, studies in Nigeria have not often reported *E. coli* as the most prevalent bacteria causing ASB or UTI [15-17]. According to Cortes-Penfield et al. [18], the epidemiological surveillance of urine from patients attending outpatient clinic can offer insights into the changing prevalence and antibiotic susceptibilities of urinary pathogens. Hence, the aim of this study was to determine the most common bacterial isolates associated with ASB in women in Lagos, Nigeria and their urinalysis profile. The study also assessed the antimicrobial susceptibility pattern of the isolated bacteria.

Materials and Methods

Study design and area

This study was a cross-sectional study. The target participants were women attending the outpatient clinic of the Lagos University Teaching Hospital (LUTH) in Lagos State, Nigeria. The study included females who were 18 years or older, did not have any symptoms of a urinary tract infection, were not currently undergoing antibiotic therapy, or had used antibiotics in the previous three weeks, and were willing to participate.

Ethical consideration

A written informed consent was collected from all participants. The confidentiality of the participants was maintained as no identifying information was collected from them. Ethical approval was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research.

Sample collection

All consenting women were instructed on the collection of clean-catch mid-stream urine after informed consent had been given. The urine collection involved cleaning the vulva with clean water followed by voiding the first flow of urine. Then about 10 ml of two consecutive mid-stream urine samples were collected into sterile universal bottles from each participant. The age and information on whether they have had any history of urinary tract infection or sexually transmitted infection were also recorded for each participant. Urine samples were analyzed within one hour of sample collection.

Sample analysis

Tests for significant bacteriuria were done using the microscopic methods as previously described [19,20]. All urine samples having 2 or more bacteria, consistently distributed per oil immersion field on the examination of 20 or more fields, were regarded as positive for ASB [19,20]. Primary isolation of bacteria from ASB positive urine samples was done on Cystine-Lactose Electrolyte Deficient (CLED) and chocolate agar, and single colonies with different morphological characteristics were sub-cultured on MacConkey, Nutrient, and Mannitol salt agar.

Bacterial isolates were identified on the basis of colony morphology and using gram staining and biochemical tests such as Beta-glucuronidase, catalase, citrate, urease, indole, oxidase, DNase, and lysine decarboxylase, amongst others, using methods as described in the District Laboratory Practice in Tropical Countries [21].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on the pure isolates using the Kirby Bauer disc diffusion method and interpreted according to the Clinical and Laboratory Standard Institute guidelines [22]. Inoculums at a 0.5 McFarland standard equivalent were cultured on Mueller Hinton Agar and subjected to antimicrobial susceptibility testing using the antibiotics gentamicin 10 µg, ofloxacin 5 µg, erythromycin 5 µg, amoxicillin-clavulanate 30 µg, cloxacillin 5 µg, ceftazidime 30 µg, cefuroxime 30 µg, ampicillin 10 µg, tetracycline 25 µg, trimethoprim/sulfamethoxazole 25 µg, imipenem 10 µg, cefoxitin 30 µg, nalidixic acid 10 µg, nitrofurantoin 300 µg, and vancomycin 30 µg. The zone of inhibition was interpreted as sensitive or resistant using the CLSI criteria.

Urinalysis

Urine analysis was carried out using a commercial biochemical reagent strip; the Medi-Test urine test strips (Macherey-Nagel, Germany). The test strip measures the biochemical parameters of blood, urobilinogen, bilirubin, protein, nitrite, ketones, glucose, pH, density, and leukocytes in urine.

Statistical analysis

Data analysis was done using both descriptive and inferential statistics at the 95% confidence level using SPSS version 26.0. Tests of significance were done with χ^2 for the type of bacteria isolated and asymptomatic bacteriuria and for the type of bacteria isolated and history of UTI. The χ^2 was also used to check for the association between the urinalysis profile and type of bacteria isolated.

Results and Discussion

Bacteria distribution in asymptomatic bacteriuria

A total of 162 women who met the inclusion criteria agreed to participate in this study. Of these women, only 45 (27.8%) were positive for asymptomatic bacteriuria. However, 48 bacterial isolates were recovered from these 45 women, with 3 women having dual infection with *E. coli* and *Staphylococcus aureus*, another 3 having dual infection with *E. coli* and *Streptococcus* species, and *Staphylococcus aureus* and *Klebsiella* species. Figure 1 shows the bacterial distribution in the 45 women with ASB. Thirteen different species of bacteria were isolated from the urine specimen, of which 20 were gram-positive and 28 were gram-negative. The frequency and profile of the bacteria isolated are presented in Table 1. Seventeen out of the 48 isolated bacteria were staphylococci species. Statistical analysis showed that there was a significant isolation of staphylococci in cases with significant bacteriuria ($p=0.0001$).

Of the 45 patients with significant bacteriuria and bacterial culture

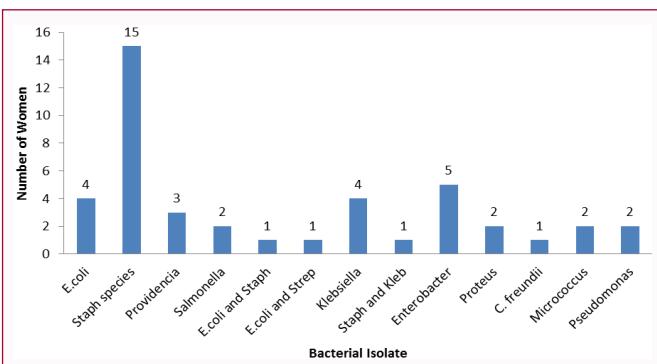


Figure 1: Bacteria distribution in women with asymptomatic bacteriuria.

Table 1: Profile and frequency of bacteria isolated from women with asymptomatic bacteriuria.

S/No	Gram positive isolates	No Isolated	Percentage
1	<i>Staphylococcus</i> species	17	35.42
2	<i>Streptococcus</i> species	1	2.08
3	<i>Micrococcus</i>	2	4.17
Gram negative isolates			
4	<i>Escherichia coli</i>	6	12.5
4	<i>Enterobacter cloacae</i>	5	10.42
4	<i>Klebsiella aerogenes</i>	5	10.42
6	<i>Providencia rettgeri</i>	3	6.25
7	<i>Proteus mirabilis</i>	2	4.17
8	<i>Pseudomonas aeruginosa</i>	2	4.17
10	<i>Salmonella enterica</i>	2	4.17
11	<i>Citrobacter freundii</i>	1	2.08
12	<i>Serratia marcescens</i>	1	2.08
13	<i>Yersinia</i> species	1	2.08

growth, 24 (53%) had a history of urinary tract infection and/or sexually transmitted infection. However, staphylococci species were isolated from only 25% of the 24 participants with a history of UTI. At the same time, there was no statistically significant relationship between the history of UTI/STI and the staphylococci-associated asymptomatic bacteriuria ($p=0.057$). The 17 staphylococcal species were further characterized as *Staphylococcus aureus* 8 (47.1%), *Staphylococcus saprophyticus* 5 (29.4%) and other coagulase-negative staphylococci 4 (23.5%).

Escherichia coli are the most commonly reported cause of asymptomatic bacteriuria. However, other gram-negative organisms such as *Klebsiella* species, *Citrobacter* species, *Proteus* species, *Providencia* species, and *Pseudomonas aeruginosa* have been

associated with asymptomatic bacteriuria in this particular order [1]. In this study, *Staphylococcus aureus* was the most common bacteria isolated associated with ASB. This was followed by *E. coli*, *Staphylococcus saprophyticus*, other coagulase-negative staphylococci, and then *Enterobacter cloacae* and *Klebsiella aerogenes*. Our result corroborates the study by another report from Nigeria, which also reported *Staphylococcus aureus* as the most common bacteria isolated from ASB in pregnant women [16]. Similarly, *Staphylococcus aureus*, followed by *Escherichia coli* and *Staphylococcus saprophyticus*, was also the most predominant uropathogen isolated from people with urinary tract infection in another study [15]. In a previous study, we also found staphylococci species as significant bacteria causing urinary tract infections in pregnant women [23].

The frequency and profile of gram negative bacterial isolation associated with ASB was similar to that previously reported [1,14]. In our study, the most prevalent gram-negative bacteria causing ASB were *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella aerogenes*, *Providencia rettgeri*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella enterica* while the most prevalent gram-positive bacteria causing ASB were *Staphylococcus aureus*, *Staphylococcus saprophyticus*, other coagulase-negative staphylococci, and *Micrococcus* species.

Sensitivity pattern of urinary isolates

All the organisms, both gram-positive and gram-negative, were susceptible to nitrofurantoin and imipenem. In addition to this, all the gram positive bacteria were susceptible to vancomycin, while all the gram negative bacteria were susceptible to gentamicin. High resistance to ampicillin, augmentin, and tetracycline was recorded. Cefoxitin was used as a measure for methicillin resistance and 6 (75%) out of the 8 *Staphylococcus aureus* and 1 (25%) out of the 5 *Staphylococcus saprophyticus* were methicillin resistant. The antibiotic susceptibility pattern of the isolated bacteria is presented in Table 2.

The guidelines of the Infectious Diseases Society of America

Table 2: Sensitivity pattern of the urinary isolates.

Gram Positive Isolates	No Isolated	CAZ (%)	CRX (%)	GEN (%)	OFL (%)	AUG (%)	IMP (%)	TET (%)	NIT (%)	CXC (%)	CEF (%)	ERY (%)	VAN (%)
<i>Staphylococcus aureus</i>	8	25	37.5	100	50	0	100	25	100	25	25	75	100
<i>Staphylococcus saprophyticus</i>	5	60	40	100	60	20	100	40	100	40	80	80	100
other CoNS	4	75	25	100	75	25	100	50	100	50	100	75	100
<i>Streptococcus</i> species	1	100	100	100	100	0	100	0	100	100	100	100	100
<i>Micrococcus</i>	2	100	50	50	100	0	100	50	100	50	100	50	100
Gram Negative Isolates		CAZ (%)	CRX (%)	GEN (%)	OFL (%)	AUG (%)	IMP (%)	TET (%)	NIT (%)	AMP (%)	NAL (%)	TS (%)	CPR (%)
<i>Escherichia coli</i>	6	50	33	100	50	17	100	0	100	0	67	83	67
<i>Enterobacter cloacae</i>	5	60	20	100	60	0	100	80	100	0	60	80	80
<i>Klebsiella aerogenes</i>	5	100	20	100	60	20	100	20	100	0	80	80	80
<i>Providencia rettgeri</i>	3	100	33	100	67	33	100	0	100	0	100	100	100
<i>Proteus mirabilis</i>	2	100	50	100	100	0	100	0	100	0	100	100	100
<i>Pseudomonas aeruginosa</i>	2	100	0	100	100	0	100	0	100	0	100	50	100
<i>Salmonella enterica</i>	2	100	0	100	100	50	100	0	100	0	100	100	0
<i>Citrobacter freundii</i>	1	100	0	100	100	0	100	0	100	0	100	0	100
<i>Serratia marcescens</i>	1	100	0	100	100	0	100	0	100	0	100	100	100
<i>Yersinia</i> species	1	100	0	100	100	0	100	0	100	0	100	100	100

Key: CAZ: Ceftazidime; CRX: Cefuroxime; EN: Gentamicin; OFL: Ofloxacin; AUG: Amoxycillin/Clavulanate; IMP: Imipenem; TET: Tetracycline; NIT: Nitrofurantoin; CAZ: Ceftazidime; CXC: Cloxacillin; CEF: Cefoxitin; ERY: Erythromycin; VAN: Vancomycin; AMP: Ampicillin; NAL: Nalidixic Acid; TS: Trimethoprim/Sulfamethoxazole; CPR: Ciprofloxacin

Table 3: Urinalysis Result in the 45 patients with positive culture.

Parameter	Negative/Normal (%)	Positive			
		+ (%)	++ (%)	+++ (%)	++++ (%)
Bilirubin	45 (100)	0	0	0	0
Blood	45 (100)	0	0	0	0
Density	43 (95.6)	2	0	0	0
Glucose	42 (93.3)	1	1	0	0
Leucocytes	30 (66.7)	5 (11.1)	8 (17.8)	2 (4.4)	0
Nitrites	18 (40)	27 (60)			
Protein	40 (88.9)	5 (11.1)	0	0	0
Urobilinogen	45 (100)	0	0	0	0

Key: Blood: Normal, ca. 5–10 Ery/ μ L (+), ca. 50 Ery/ μ L (++) , ca. 250 Ery/ μ L (+++); Density: Normal (1.000 - 1.025); Glucose: 50 mg/dL (+), 150 mg/dL (++) , 500 mg/dL (+++), \geq 1000 mg/dL (++++); Leukocytes: neg • ca. 25 Leuco/ μ L (+), ca. 75 Leuco/ μ L (++) , ca. 500 Leuco/ μ L (+++); Protein: Negative, 30 mg/dL(+), 100 mg/dL(++) , 500mg/dL(+++); Urobilinogen: Normal, 2 mg/dL(+), 4 mg/dL (++) , 8 mg/dL (+++), 12 mg/dL (++++)

recommend that treatment of ASB should not be done in the general population but should only be prioritized in pregnant women and patients undergoing endo-urological surgeries [1,12,24]. Even in this population, treatment should be guided by culture and sensitivity results [10,25]. Antibiotics such as amoxicillin, amoxicillin/clavulanate, cefuroxime, cephalexin, and nitrofurantoin are considered safe in pregnancy and are typically recommended for the treatment of ASB in pregnant women [26,27]. Apart from the beta-lactams and nitrofurantoin, other antibiotics such as vancomycin, metronidazole, clindamycin, and fosfomycin are also generally considered safe in pregnancy [26]. In this study, high levels of resistance were recorded for amoxicillin/clavulanate and cefuroxime, which signifies that these antibiotics will not be effective in the treatment of ASB if used in pregnancy. Interestingly, all the bacteria isolated from this study were susceptible to nitrofurantoin. Hence, our study supports the use of nitrofurantoin in the treatment of ASB in the necessary populations. The gram positive bacteria were also all susceptible to vancomycin and can be recommended to pregnant women with ASB. All the isolates in the study were also susceptible to imipenem. However carbapenems should be used with caution in pregnancy and should be used only if there are no options for beta-lactams or cephalosporin's [26].

Urinalysis profile

The urinalysis profile of the participants with ASB is presented in Table 3. All the women had normal levels of blood, urobilinogen, bilirubin, and ketones. A positive nitrite result served as an indicator of significant bacteriuria, with positive nitrite results found in 60% of participants with significant bacteriuria. The patients (4.4%) showed the presence of glucose in the urine. The ages of the women with glucose in the urine were over 60 years old, and this can be indicative of diabetes in these women. The presence of glucose and proteins in the urine was not significantly associated with significant bacteriuria.

Fifteen of the 45 women with significant bacteriuria showed the presence of leucocytes in their urine. Of these 15 women, staphylococci were isolated from 12 of them. There was a statistically significant association between the presence of staphylococci and leukocyturia (the chi-square statistic is 17.0641, p=0.000036). There was, however, no significant association between leukocyturia and past history of UTI (chi-square statistic =1.6071, p=0.205).

Bacterial isolation from two consecutive voided urine specimens

is recommended as the gold standard for identifying bacteriuria in women [1,24]. However, some laboratories use urinalysis results for diagnosing urinary tract infections. The use of such non-culture urine tests is not sensitive and specific for the detection of bacteria in urine and should not be used to replace urine culture for the identification of significant bacteriuria. However, some positive parameters in the urinalysis test can be further investigated with urine culture to establish significant bacteriuria. In this study, a positive nitrite result was significantly associated with significant bacteriuria. Other studies have also reported that nitrite positivity in the urine can serve as an indication of significant bacteriuria [17]. Nonetheless, the use of nitrite positivity to infer significant bacteriuria is not encouraged. This is because some bacteria that can cause urinary infections are nitrite negative and it also takes about 4 h to 6 h for bacteria to convert nitrate to nitrite, leading to false negative results [28,29]. Using nitrite positivity as an indication of significant bacteriuria will mean that such false negative results will be missed.

In this study, the presence of leucocytes in the urine was not a significant indicator of significant bacteriuria; however, it was a significant indicator of staphylococcal infection. Leukocyturia is generally defined as the presence of leukocytes in the urine and can be due to urinary infections or non-infectious factors [30]. High leukocyte levels in the urine indicative of leukocyturia can be found in patients with some conditions such as chronic renal failure, heart failure, and diabetes mellitus [30]. The two women with high glucose levels in their urine also had detectable urine leukocyte levels. This may further indicate diabetes mellitus in these women. A study reported that an increase in urine leukocyte levels can predict the transition from asymptomatic bacteriuria to symptomatic urinary tract infection in women with recurring urinary tract infections [31]. However, there was no significant association between leukocyturia and a history of urinary tract infection in this study.

Conclusion

In conclusion, staphylococci species appear to be the new most common cause of asymptomatic bacteriuria and, by extension, urinary tract infection in Lagos, Nigeria. Nitrofurantoin and vancomycin are effective in the treatment of asymptomatic bacteriuria in pregnant women. The use of urinalysis results is not sufficient in establishing significant bacteriuria.

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