

**ANTIBIOTICS SUSCEPTIBILITY PROFILE AND PREVALENCE OF GRAM-
NEGATIVE UROPATHOGENS FROM ASYMPTOMATIC BACTERIURIA
AMONG FEMALE STUDENTS IN A UNIVERSITY IN NORTHERN NIGERIA**

ABSTRACT

Asymptomatic bacteriuria among healthy female students is a common occurrence that is frequently ignored and this is attributed to the fact that Pre-menopausal, non-pregnant women with asymptomatic bacteriuria experience no adverse effects and usually will clear their bacteriuria spontaneously. However, these women are more likely to experience subsequent symptomatic UTI than women who do not have asymptomatic bacteriuria. The aim of this study is to determine the incidence of asymptomatic bacteriuria among female students of Ahmadu Bello University (A.B.U), Zaria Main Campus.

Methodology: A total of 400 midstream clean-catch urine samples were analyzed using standard microbiological methods. Organisms were isolated from positive urine samples and the isolates were identified using Microgen™ GNA-ID kit. Antibiotic susceptibility was carried out on the isolates using the modified Kirby-Bauer disc diffusion test method.

Result: Bacteriological analysis of the urine samples revealed a 16% (63/400) asymptomatic bacteriuria among female students in A.B.U main campus with a total of 148 bacteria isolates. The most prevalent bacteria were *Klebsiella* species and *Acinetobacter* species (19.59%), followed by *Enterobacter* species (17.57%) and *Escherichia* species (11.49%). A high incidence of resistance to Tetracycline (74%) and Cephalosporin (78%) was observed (Fig. 2).

Conclusion: This study showed that there is an incidence of asymptomatic bacteriuria 16% (63) among healthy female students in A.B.U main campus and these isolates show a high resistance to Tetracycline (74%) and Cephalosporin (78%) antibiotics.

28 INTRODUCTION

29 Urine formed in the kidney is a sterile fluid. It can serve as a good culture medium for
30 multiplication of bacteria, hence small amount of bacteria may be found in the urine of many
31 healthy people. This could occur as a result of introduction of bacteria into the urinary tracts
32 as a result of ascension (Harvey and Zieve, 2013), which are usually considered to be
33 harmless. However, certain level of bacteria can mean that the bladder, urethra or kidney is
34 infected and colonized (Omonigbo *et al.*, 2001; Najar *et al.*, 2009).

35 Urinary tract infection (UTI) may be defined as a condition in which bacteria are established
36 and multiplying within the urinary tract (Najar *et al.*, 2009). UTI can be grouped into
37 asymptomatic and symptomatic cases based on the pathogenesis of the infection and it is a
38 disease that commonly affects people of all age groups and sexes (Ojo *et al.*, 2004).

39 Bacteriuria is the presence of bacteria in urine not due to contamination from urine sample
40 collection (Colgan *et al.*, 2006). “Asymptomatic bacteriuria, (ASB)” or asymptomatic urinary
41 infection is the isolation of actively multiplying bacteria of a specified quantitative count in
42 an appropriately collected urine specimen obtained from a person without symptoms or signs
43 of urinary tract infection such as burning sensation during urination or frequent urination
44 (Imade *et al.*, 2010; Nicolle, 2014). The usual quantitative definition for the diagnosis of
45 asymptomatic bacteriuria is $\geq 10^5$ cfu/mL in urine specimens (Nicolle *et al.*, 2005).

46 Asymptomatic bacteriuria occurs more frequently in females as compared with males by
47 virtue of the shortened urethra (Piatti *et al.*, 2008; Sheffield and Cunningham, 2005; Olaitan,
48 2006) and it is a major criterion of urinary tract infection (Nurullaev, 2004). Implicated
49 microorganisms include *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Enterococcus*
50 species, and group B *Streptococcus*. *Escherichia coli* is the most commonly isolated

51 organism from patients with asymptomatic bacteriuria (Ezeome *et al.*, 2006; Oli *et al.*, 2010;
52 Shoff, 2014).

53 In healthy women, the prevalence of bacteriuria increases with age, from about 1% in females
54 aged 5 to 14 years to more than 20% in women above 80 years of age (Nicolle, 2003). Pre-
55 menopausal, non-pregnant women with asymptomatic bacteriuria experience no adverse
56 effects and usually will clear their bacteriuria spontaneously due to strong immunity;
57 however, these women are more likely to experience subsequent symptomatic UTI than
58 women who do not have asymptomatic bacteriuria (Hooton *et al.*, 2000).

59 One of the causes of asymptomatic bacteriuria is the normal flora of the gut, which ascends
60 up the urethra into the bladder and potentially the kidneys. This can happen during such
61 activities as sexual intercourse or wiping towards the urethra after using the toilet (Harvey
62 and Zieve, 2013; Nicolle, 2014). Most female students found among a University population
63 are sexually active as such poor hygiene can lead to bacteria colonization of the urethra
64 (Nicolle *et al.*, 2005).

65 In the past studies focused more on ASB in pregnant women due to the associated risk
66 (Akerele *et al.*, 2001; Onifade *et al.*, 2005; Okonko *et al.*, 2009; Taiwo *et al.*, 2009; Omole-
67 Ohonsi and Nwokedi, 2009; Oli *et al.*, 2010) hence, it is important to assess the colonization
68 of the urinary tract by bacteria among non-pregnant women.

69 **MATERIALS AND METHODS**

70 **Study area**

71 Ahmadu Bello University Main Campus, Zaria Nigeria is situated in Samaru community,
72 along Sokoto Road on Latitude: 11°9'2.16" and Longitude: 7°39'17.28". The map is as shown
73 in Figure 1.



Fig 1: Google map of Ahmadu Bello University, Zaria.

Sample Collection and Transportation

Midstream, clean-catch urine samples were collected into sterile containers from asymptomatic female students of the Ahmadu Bello University main campus Zaria, Nigeria. Proper instruction on how to collect urine sample was given to the students. These were immediately sent to the laboratory in an ice pack for processing using the standard microbiological methods and examined immediately. Analysis on collected urine was carried out within 6 hours of collection.

Microscopy and Determination of Significant Bacteriuria

A presumptive test method as described by Vandepitte *et al.* (2003) 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 bacteria cells per oil-immersion field or more bacteria per milliliter in the specimen were selected as having 10^5 cfu/ml (positive).

Isolation and Identification of organisms

The Calibrated loop technique was used to confirm samples that were positive for significant bacteriuria from the presumptive test and for isolation of organism (Chessbrough 2000). A

93 calibrated metal loop was used to transfer 0.001ml of urine to the culture medium. This was
94 streaked on the MacConkey agar, Eosin Methylene Blue agar, Nutrient agar and CLED
95 (Cystine-Lactose-Electrolyte-Deficient) agar media and the plates were incubated overnight
96 at 35°C. The approximate number of bacteria per ml of urine was estimated as described by
97 Chessbrough (2000). The Nutrient agar and CLED Agar plates were used for colony counting
98 while the MacConkey agar and Eosin Methylene Blue agar plates were used as selective
99 media for Enterobacteriaceae. The number of colonies on the agar plate was counted using a
100 colony counter and multiplied by 1000 (10^3). A positive urine culture was defined as positive
101 if $\geq 10^5$ colony forming units (CFU) per mL are observed (Stamm and Hooton, 1993;
102 Cheesbrough, 2000) while cultures with no growth of bacteria were considered to be
103 negative.

104 Morphological characteristics such as size, shape, and color of these colonies helped to
105 identify the types of bacteria present in the urine sample (Cowan 1974; Collee and Miles,
106 1989). The bacterial isolates were identified using MicrogenTM GNA-ID kit following the
107 manufacturer's instructions. A pure 18-24 hour culture of the bacterial isolate to be identified
108 was used. A single colony was emulsified from an 18-24 hour culture in 3mL sterile 0.85%
109 saline for the GN A micro-well test strip and mixed thoroughly. Using a sterile Pasteur's
110 pipette, 3-4 drops (approximately 100µL) of the bacterial suspension was added to each well
111 of the micro-well test strip(s). After inoculation, wells 1,2 3 and 9 (GN A micro-well test
112 strip counting from the tabbed end) were overlaid with 3-4 drops of mineral oil. The top of
113 the micro-well test strip(s) were sealed with the adhesive tape removed earlier and incubated
114 at 35-37°C. The GN A micro-well test strips were read after 18-24 hours incubation. All
115 positive reactions were read with the aid of the color chart and the results were recorded on
116 the forms provided. The appropriate reagents were added to the required micro-wells. The

Microgen Identification System Software (MID-60) was used for identification of the organisms to specie levels.

Antibiotic 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 (2000) and CLSI (2014). Antibiotics tested were obtained from Oxoid Ltd and include; Pefloxacin (5µg), Norfloxacin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Amoxicillin-clavulanic acid (30µg), Nitrofurantoin (300µg), Trimethoprim/Sulfamethoxazole (30µg), Gentamicin (10µg), Tetracycline (30µg) and Ceftriaxone (30µg). A suspension of overnight growth of each isolate on Nutrient agar plates was standardized. With the aid of a wire loop, a colony was picked and transferred into the tube of sterile normal saline and the turbidity was compared with 0.5 McFarland standards corresponding to approximately 1.5×10^8 cfu/ml. Suspension of the isolates was inoculated on Mueller 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 a sterile forcep. Within 30 minutes of applying the discs, plates were inverted and incubated at 35°C for 16–18 hours. After overnight incubation, plates were examined and the diameter of each zone of growth inhibition around the discs was measured in mm, using a ruler on the underside of the plate. Using the Interpretative Chart (CLSI, 2014), 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 (Magiorakos *et al.*, 2012).

RESULTS

Out of the 400 urine samples cultured for asymptomatic bacteriuria, 16% (63) were positive for significant bacteriuria (10^5 cfu/ml) among the female students while 84% (337) of the studied population did not show significant bacteriuria. A total of 148 isolates were obtained from the asymptomatic bacteriuria positive urine samples with mixed growth of organisms.

Table 1: Incidence of asymptomatic bacteriuria among female students

PARAMETERS	NUMBER
Number of Sample Screened	400
Number with Significant Bacteriuria	63
Number with no Significant Bacteriuria	337
Number of Isolated Bacteria	148

Female students within age bracket 20-24 years showed the highest incidence of asymptomatic bacteriuria 41 (65%) as shown in Table 2.

Table2: Incidence of ASB in relation to age distribution of female students

Age group (years)	No of Samples	No. Positive (%)
15-19	74	14 (22)
20-24	272	41 (65)
25-29	49	8 (13)
30-34	5	0 (0)
Total	400	63 (16)

Key: No-Number

The most prevalent organisms isolated from asymptomatic subjects in this study were *Klebsiella* species (19.59%) and *Acinetobacter* species (19.59). This was followed by *Enterobacter* species (17.57%), *Escherichia coli* (11.49%), *Salmonella* species (9.46%) and *Serratia* species (8.78%) as shown in table 3 below.

158 **Table 3: Distribution of Isolates**

ISOLATES	Number (%)
<i>Klebsiella</i> spp	29 (19.59)
<i>Acinetobacter</i> spp	29 (19.59)
<i>Enterobacter</i> spp	26(17.57)
<i>Escherichia coli</i>	17 (11.49)
<i>Salmonella</i> spp	14 (9.46)
<i>Serratia</i> spp	13(8.78)
<i>Citrobacter</i> spp	8 (5.40)
<i>Proteus</i> spp	6 (4.05)
<i>Yersinia</i> spp	3 (2.03)
<i>Providencia</i> spp	2 (1.35)
<i>Shigella</i> spp	1 (0.68)
Total	148 (100)

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160

161 **Table 4: Isolates obtained using Microgen identification kit (Microgen™ GNA-ID)**
162 **to specie level**

ISOLATES	Number (%)
<i>Acinetobacter baumannii</i>	20 (13.51)
<i>Klebsiella oxytoca</i>	17 (11.49)
<i>Escherichia coli</i>	17 (11.49)
<i>Serratia marcescens</i>	12 (8.12)
<i>Klebsiella pneumoniae</i>	11 (7.43)
<i>Enterobacter agglomerans</i>	11 (7.43)
<i>Salmonella arizonae</i>	10 (6.76)
<i>Enterobacter gergoviae</i>	9 (6.08)
<i>Acinetobacter lwoffii</i>	9 (6.08)
<i>Proteus mirabilis</i>	6 (4.05)
<i>Enterobacter sakazaki</i>	3 (2.03)
<i>Enterobacter aerogenes</i>	3 (2.03)
<i>Yersinia enterocolitica</i>	3 (2.03)
<i>Citrobacter diversus</i>	3 (2.03)
<i>Citrobacter freundii</i>	3 (2.03)
<i>Proteus vulgaris</i>	2 (1.35)
<i>Providencia stuartii</i>	2 (1.35)
<i>Salmonella paratyphi</i>	1 (0.68)
<i>Salmonella choleraesuis</i>	1 (0.68)
<i>Salmonella</i> spp	1 (0.68)
<i>Salmonella gallinarum</i>	1 (0.68)
<i>Klebsiella ozaenae</i>	1 (0.68)
<i>Serratia liquefaciens</i>	1 (0.68)
<i>Shigella</i> serogroup A,B& C	1 (0.68)

Total	148 (100)
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The asymptomatic bacteriuria isolates showed high resistance to Tetracycline and Ceftriaxone. Gentamicin, Ciprofloxacin, Pefloxacin, Levofloxacin and Norfloxacin had great antimicrobial activity against the isolates as shown in table 5 below.

Table 5: Resistance Profile of Bacteria Isolates to Antibiotics

ISOLATE	RESISTANCE									
	CIP (5 µg)	AMC (20 µg)	SXT (25 µg)	PEF (5 µg)	CRO (30 µg)	TE (30 µg)	CN (10 µg)	LEV (5 µg)	F (300 µg)	NOR (10 µg)
<i>Serratia spp</i>	7.7	30.8	38.5	7.7	100.0	76.9	0.0	0.0	53.8	0.0
<i>Escherichia spp</i>	11.8	23.5	23.5	5.9	88.2	82.3	11.8	5.9	41.2	5.9
<i>Proteus spp</i>	0.0	12.5	0.0	0.0	62.5	100.0	0.0	0.0	87.5	0.0
<i>Klebsiella spp</i>	3.4	20.7	13.8	0.0	69.0	65.5	0.0	3.4	62.1	3.4
<i>Enterobacter spp</i>	7.7	38.5	11.5	15.4	69.2	69.2	0.0	3.8	46.1	11.5
<i>Salmonella spp</i>	0.0	35.7	42.9	14.3	71.4	71.4	14.3	0.0	57.1	7.1
<i>Acinetobacter spp</i>	10.3	13.8	10.3	6.9	86.2	65.5	0.0	6.9	58.6	10.3
<i>Yersinia spp</i>	0.0	33.3	0.0	0.0	33.3	33.3	0.0	0.0	33.3	0.0
<i>Providencia spp</i>	0.0	0.0	0.0	50.0	100.0	50.0	0.0	0.0	100.0	50.0
<i>Citrobacter spp</i>	0.0	16.7	16.7	0.0	83.3	100.0	16.7	16.7	50.0	0.0
<i>Shigella spp</i>	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0

Keys: CIP= Ciprofloxacin, AMC= Amoxicillin/Clavulanic Acid, SXT= Co-trimoxazole, PEF= Pefloxacin, CRO= Ceftriaxone, TE= Tetracycline, CN= Gentamicin, LEV= Levofloxacin, F= Nitrofurantoin, NOR= Norfloxacin

Based on the antibiotic classes, 78.4% of the isolates were resistant to Cephalosporin, 74.3% of the isolates were resistant to Tetracycline, 24.3% of the isolates were resistant to Penicillin, 54.1% showed resistance to Nitrofurans, 16.9% showed resistance to Co-trimoxazole and only 3.4% of the isolates showed resistance to Aminoglycoside.

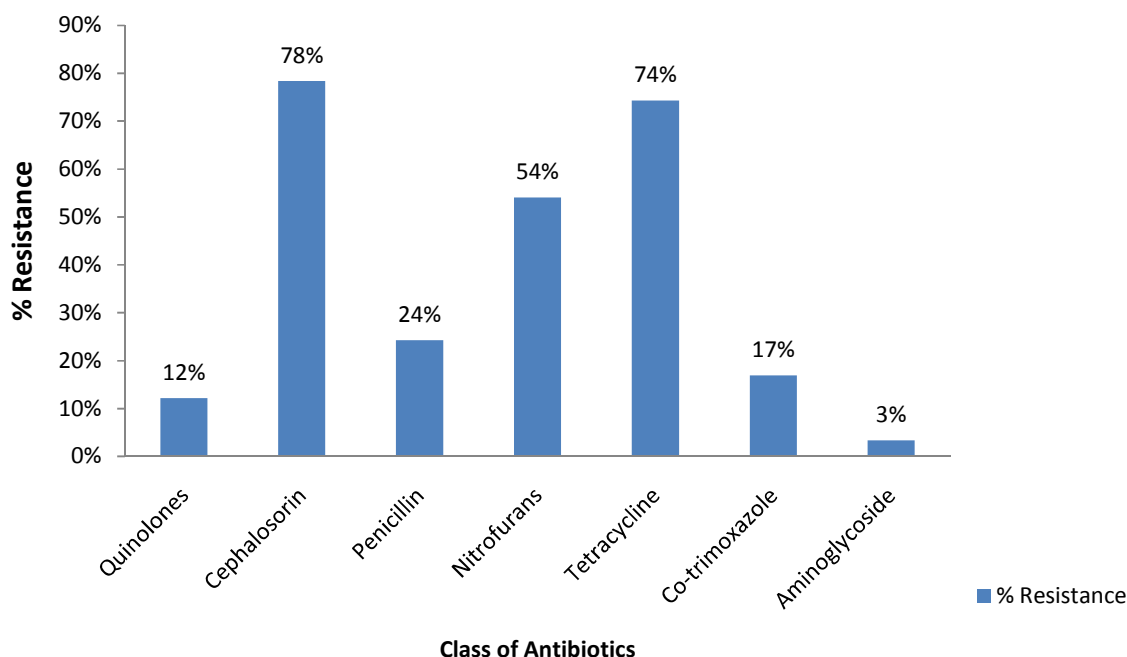


Fig 2: Resistance of asymptomatic bacteriuria isolates to antibiotics classes

DISCUSSION

This study showed a 16% incidence rate of asymptomatic bacteriuria amongst the sampled population of 400 female students who reside in the female hostels of the Ahmadu Bello University Main Campus, Zaria (Table 1). This level of prevalence is comparable to the results of other researchers; Olaitan (2006), who recorded an incidence rate of 10% of asymptomatic bacteriuria among female students in Lagos state University, Nigeria. Ettehad *et al.* (2007) reported an incidence of 9.2% in a study carried out among 207 female students in a Tertiary institution in Iran, this is also similar to the findings of Wogu and Ogbebor (2011), who found out that asymptomatic bacteriuria, was present in 12.78% of 118 female students sampled in Benin City, South-south-Nigeria. The low incidence of asymptomatic bacteriuria among the young female population may be attributed to the ability of young healthy non-pregnant women to clear asymptomatic bacteriuria due to strong immunity (Colgan *et al.*, 2006).

This value is higher than 5% incidence bacteriuria among students of secondary schools (Omer and Ahmed, 1992) and females of 14-17 year of age with 3.3% bacteriuria (Brumffitet *al.*, 1987). The higher value obtained as compared to what is obtained from secondary school students could be possibly due to increase in sexual activities among the University age group and /or increased age. In young women asymptomatic bacteriuria has been said to be strongly associated with sexual activities (Nicole *et al.*, 2005).

The organisms usually associated with asymptomatic bacteriuria are members of the gastrointestinal and vaginal flora. These include Gram-negative organisms, i.e. *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Providencia* and *Morganella* species (Maranchie *et al.*, 1997; Walker *et al.*, 2000; Nicolle, 2001; Tal *et al.*, 2005). *Serratia* species, *Pseudomonas aeruginosa* and *Acinetobacter* species are also implicated in asymptomatic bacteriuria (Muhammad *et al.*, 2002; Raza *et al.*, 2011). This agrees with the result of this study as most of these organisms were isolated from asymptomatic bacteriuric subjects (Table 3). It has been observed that bacteriuria in young girls and women are preceded by colonisation of the vaginal introitus by the specific species of Enterobacteriaceae that produces the infection (Olaitan, 2006). 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 (Olaitan, 2006).

This study revealed two organisms to be most prevalent among female students of Ahmadu Bello University Main Campus, Zaria, Nigeria; these include *Acinetobacter* species (19.59%) and *Klebsiella* species (19.59%) while *Escherichia coli* 11.49% and *Proteus* species 5.40% (Table 3). Although earlier researchers reported that *Escherichia coli* were often the most common isolated organism in asymptomatic bacteriuria, (Onifade *et al.*, 2005; Turpin *et al.*, 2007; Obiogbolu *et al.*, 2009; Andabati and Byamugisha, 2010), recent reports have shown that organisms such as *Staphylococcus* species, *Klebsiella* specie and *Proteus* species are

taking the position of *Escherichia coli* (Ogbolu *et al.*, 2006; Amadi *et al.*, 2007; Ajayi *et al.*, 2012). The result obtained agrees with the report of Okon *et al.* (2012), who isolated *Klebsiella* species as the most prevalent organism from asymptomatic bacteriuria. The high prevalence of *Acinetobacter* species isolated in this study show a close similarity to the findings of Thakur *et al.* (2003) who recorded a high prevalence of *Acinetobacter* species with a prevalence rate of 15% this might be attributed to some species of *Acinetobacter* being common commensals of the skin, throat and secretions of healthy people (Kuo and Chen, 2014). *Acinetobacter* species has been reported to cause urinary tract infection (Antunes *et al.*, 2011), hence high incidence of *Acinetobacter* species in the urinary tract may lead to subsequent urinary tract infection (UTI).

A high incidence of resistance to Tetracycline (74%) and Ceftriaxone (78%) was observed in this study among the asymptomatic bacteriuria isolates. This is in agreement with the studies carried out by Ngwai *et al.* (2010) in Bayelsa State, Nigeria and Mansour *et al.* (2009) in Iran who reported a high resistance rate of asymptomatic bacteriuria isolates to tetracycline. This could be as a result the irrational and indiscriminate use of these groups of antibiotics (Okeke *et al.*, 2005). Less resistance was observed with Gentamicin and Quinolone. The result obtained is in agreement with a study carried out in Maiduguri, Nigeria by Okon *et al.* (2012) and in Benin, Nigeria by Akerele *et al.* (2001). They reported less resistance to the quinolone group of antibiotics.

CONCLUSION

There is an incidence of asymptomatic bacteriuria among the female students of Ahmadu Bello University Main Campus. Enterobacteriaceae were the major organisms isolated from asymptomatic bacteriuria subjects and the isolates showed high resistance to Tetracycline and Ceftriazone.

RECOMMENDATION

This study therefore recommends proper education of the populace on the consequence of asymptomatic bacteriuria among young female, the need for proper hygiene especially among sexually active young female population so as to reduce the incidence of asymptomatic bacteriuria. It is also important to curb the causes of antibiotic resistance.

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