

Original Research Article

**ANTIBIOTICS SUSCEPTIBILITY PROFILE AND PREVALENCE OF GRAM-
NEGATIVE UROPATHOGENS/URINARY ISOLATES 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.(Not detail enough)

Results: 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* spp species and *Acinetobacter* spp species (19.59%), followed by *Enterobacter* spp (17.57%) and *Escherichia* spp (11.49%). A high incidence of resistance to Tetracycline (74%) and Ceftriaxone (78%) was observed. This study showed that there is an incidence of asymptomatic bacteriuria among healthy female students in A.B.U main campus.

Conclusion:

INTRODUCTION

- 25 Urine formed in the kidney is a sterile fluid. It can serve as a good culture medium for
- 26 multiplication of bacteria, hence small amount of bacteria may be found in the urine of many

27 healthy people. This **can** occur as a result of introduction of bacteria into the urinary tracts as
 28 a result of ascension (Harvey and Zieve, 2013), which are usually considered to be harmless.
 29 However, certain level of bacteria can mean that the bladder, urethra or kidney is infected and
 30 colonized (Omonigbo *et al.*, 2001; Najar *et al.*, 2009).

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

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

42 Asymptomatic bacteriuria occurs **reliably** more frequently in females as compared with males
 43 by virtue of the shortened urethra (Piatti *et al.*, 2008; Sheffield and Cunningham, 2005;
 44 Olaitan, 2006) and it is a major criterion of urinary tract infection (Nurullaev, 2004).
 45 Implicated microorganisms include *Enterobacteriaceae*, *Pseudomonas aeruginosa*,
 46 *Enterococcus* species, and group B *Streptococcus*. *Escherichia coli* is the most commonly
 47 isolated organism from patients with asymptomatic bacteriuria (Ezeome *et al.*, 2006; Oli *et*
 48 *al.*, 2010; Shoff, 2014).

49 In healthy women, the prevalence of bacteriuria increases with age, from about 1% in females
 50 aged 5 to 14 years to more than 20% in women above 80 years of age (Nicolle, 2003). Pre-

51 menopausal, non-pregnant women with asymptomatic bacteriuria experience no adverse
52 effects and usually will clear their bacteriuria spontaneously due to strong immunity;
53 however, these women are more likely to experience subsequent symptomatic UTI than
54 women who do not have asymptomatic bacteriuria (Hooton *et al.*, 2000).

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

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

65 MATERIALS AND METHODS

66 Study area

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



70

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

72 **Sample Collection and Transportation**

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

79 **Microscopy and Determination of Significant Bacteriuria**

80 A presumptive test method as described by Vandepitte *et al.* (2003) was used to determine
 81 significant bacteriuria. Using a sterile Pasteur pipette (one for each sample), one drop of well
 82 mixed, uncentrifuged urine was placed on a slide and gram-stained. The stained slide was
 83 examined under an oil-immersion lens (x600 or more) for presence or absence of bacteria.
 84 Samples with one or more bacteria cells per oil-immersion field or more bacteria per milliliter
 85 in the specimen were selected as having 10^5 cfu/ml (positive).

86 **Isolation and Identification of organisms**

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

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

Morphological characteristics such as size, shape, and color of these colonies helped to identify the types of bacteria present in the urine sample (Cowan 1974; Collee and Miles, 1989). The bacterial isolates were identified using Microgen™ GNA-ID kit.

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, 4 to 5 well isolated colonies of similar appearance were 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 one or more agents in three or more different classes of antibiotics (Magiorakos *et al.*, 2012). **State statistical package used for analysis.**

RESULTS AND DISCUSSION

Out of the 400 urine samples examined cultured for asymptomatic bacteriuria, 16% (63) prevalence rate 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.

Table 1: Incidence of asymptomatic bacteriuria among female students

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

The subjects screened for asymptomatic bacteriuria were in the age bracket of 15-34 years. Female students within age bracket 20-24 years showed the highest incidence of asymptomatic bacteriuria (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* spp species and *Acinetobacter* spp species with an isolation rate of 19.59% for both species of organisms. This was followed by *Enterobacter* spp with an isolation rate of 17.57%, *Escherichia* spp coli (11.49%), *Salmonella* spp (9.46%) and *Serratia* spp (8.78%) as shown in table 3 below.

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</i> spp	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)

145 **Table 4: Isolates obtained using Microgen identification kit (Microgen™ GNA-ID)**
146 **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 spp</i>	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 serogroup A,B& C</i>	1 (0.68)
Total	148 (100)

147

148 Antibiotic resistance profiles of the bacteria isolates from urine of asymptomatic subjects
149 showed that most of the isolates were resistant to the inhibitory activities of Tetracycline (%)
and

150 Ceftriaxone (%) as shown in Table 5 below.

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155 **Table 5: Resistance Profile of Bacteria Isolates to Antibiotics**

ISOLATE	RESISTANCE									
	(5 µg)	(30 µg)	(35 µg)	(5 µg)	(30 µg)	(30 µg)	(10 µg)	(5 µg)	(300 µg)	(10 µg)
										0 µg)
<i>Serratia spp</i>	R	R	R	R	R	R	S	S	R	S
<i>Escherichia spp</i>	R	R	R	R	R	R	R	R	R	R
<i>Proteus spp</i>	S	R	S	S	R	R	S	S	R	S
<i>Klebsiella spp</i>	R	R	R	S	R	R	S	R	R	R
<i>Enterobacter spp</i>	R	R	R	R	R	R	S	R	R	R
<i>Salmonella spp</i>	S	R	R	R	R	R	R	S	R	R
<i>Acinetobacter spp</i>	R	R	R	R	R	R	S	R	R	R
<i>Yersinia spp</i>	S	R	S	S	R	R	S	S	R	S
<i>Providencia spp</i>	S	S	S	R	R	R	S	S	R	R
<i>Citrobacter spp</i>	S	R	R	S	R	R	R	R	R	S
<i>Shigella spp</i>	S	S	S	S	R	R	S	S	S	S

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

159 DISCUSSION

160 This study showed a 16% incidence rate of asymptomatic bacteriuria amongst the sampled
 161 population of 400 female students who reside in the female hostels of the Ahmadu Bello
 162 University Main Campus, Zaria (Table 1). This level of prevalence is comparable to the
 163 results of other researchers; Olaitan (2006), who recorded an incidence rate of 10% of
 164 asymptomatic bacteriuria among female students in Lagos state University, Nigeria. Ettehad
 165 *et al.* (2007) reported an incidence of 9.2% in a study carried out among 207 female students
 166 in a Tertiary institution in Iran, this is also similar to the findings of Wogu and Ogbebor
 167 (2011), who found out that asymptomatic bacteriuria, was present in 12.78% of 118 female
 168 students sampled in Benin City, South-south-Nigeria. The low incidence of asymptomatic
 169 bacteriuria among the young female population may be attributed to the ability of young
 170 healthy non-pregnant women to clear asymptomatic bacteriuria due to strong immunity
 171 (Colgan *et al.*, 2006).

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

178 The organisms usually associated with asymptomatic bacteriuria are members of the
179 gastrointestinal and vaginal flora. These include Gram-negative organisms, i.e. *Escherichia*
180 *coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Providencia* and *Morganella* spp
181 (Maranchieet *al.*, 1997; Walker *et al.*, 2000; Nicolle, 2001; Tal *et al.*, 2005). *Serratia* spp,
182 *Pseudomonas aeruginosa* and *Acinetobacter* spp are also implicated in asymptomatic
183 bacteriuria (Muhammad *et al.*, 2002; Raza *et al.*, 2011). This agrees with the result of this
184 study as most of these organisms were isolated from asymptomatic bacteriuric subjects
185 (Table 3). It has been observed that bacteriuria in young girls and women are preceded by
186 colonisation of the vaginal introitus by the specific species of Enterobacteriaceae that
187 produces the infection (Olaitan, 2006). Adhesion property of the bacteria is an important
188 factor that mediates the ability of a bacterial species to colonise the vaginal or any mucosal
189 surface (Olaitan, 2006).

190 This study revealed two organisms to be most prevalent among female students of Ahmadu
191 Bello University Main Campus, Zaria, Nigeria; these include *Acinetobacter* spp (19.59%) and
192 *Klebsiella* spp (19.59%). Only 11.49% were *Escherichia* spp and 5.40% were *Proteus* spp
193 (Table 3). Although earlier researchers reported that *Escherichia* spp were often the most
194 common isolated organism in asymptomatic bacteriuria, (Onifade *et al.*, 2005; Turpin *et al.*,
195 2007; Obiogbolu *et al.*, 2009; Andabati and Byamugisha, 2010), recent reports have shown
196 that organisms such as *Staphylococcus* spp, *Klebsiella* spp and *Proteus* spp are taking the

position of *Escherichia* spp (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* spp as the most prevalent organism from asymptomatic bacteriuria. The high prevalence of *Acinetobacter* spp isolated in this study show a close similarity to the findings of Thakur *et al.* (2003) who recorded a high prevalence of *Acinetobacter* spp 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* spp has been reported to cause urinary tract infection (Antunes *et al.*, 2011), hence high incidence of *Acinetobacter* spp 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 which 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 AND RECOMMENDATION

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.

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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