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Antibiotics Susceptibility Profile and Prevalence of Gram-negative Uropathogens from Asymptomatic Bacteriuria among Female Students in a University in Northern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author PAE designed the study and wrote the protocol. Authors PAE and JCI collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Authors ROB and BOO supervised the study. Author PAE did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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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

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microbiological methods. Organisms were isolated from positive urine samples and the isolates were identified using MicrogenTM GNA-ID kit. Antibiotic susceptibility was carried out on the isolates using the modified Kirby-Bauer disc diffusion test method.
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 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.

Keywords: Urine; asymptomatic bacteriuria; uropathogens; antibiotics resistance.

1. INTRODUCTION

Urine formed in the kidney is a sterile fluid. It can serve as a good culture medium for multiplication of bacteria, hence small amount of bacteria may be found in the urine of many healthy people. This could occur as a result of introduction of bacteria into the urinary tracts as a result of ascension [1], which are usually considered to be harmless. However, certain level of bacteria can mean that the bladder, urethra or kidney is infected and colonized [2,3].

Urinary tract infection (UTI) may be defined as a condition in which bacteria are established and multiplying within the urinary tract [3]. UTI can be grouped into asymptomatic and symptomatic cases based on the pathogenesis of the infection and it is a disease that commonly affects people of all age groups and sexes [4].

Bacteriuria is the presence of bacteria in urine not due to contamination from urine sample collection [5]. “Asymptomatic bacteriuria, (ASB)” or asymptomatic urinary infection is the isolation of actively multiplying bacteria of a specified quantitative count in an appropriately collected urine specimen obtained from a person without symptoms or signs of urinary tract infection such as burning sensation during urination or frequent urination [6,7]. The usual quantitative definition for the diagnosis of asymptomatic bacteriuria is $\geq 10^5$ cfu/mL in urine specimens [8].

Asymptomatic bacteriuria occurs more frequently in females as compared with males by virtue of the shortened urethra [9-11] and it is a major criterion of urinary tract infection [12]. Implicated microorganisms include *Enterobacteriaceae, Pseudomonas aeruginosa, Enterococcus* species, and group B *Streptococcus*. *Escherichia coli* is the most commonly isolated organism from patients with asymptomatic bacteriuria [13-15].

In healthy women, the prevalence of bacteriuria increases with age, from about 1% in females aged 5 to 14 years to more than 20% in women above 80 years of age [16]. Pre-menopausal, non-pregnant women with asymptomatic bacteriuria experience no adverse effects and usually will clear their bacteriuria spontaneously due to strong immunity; however, these women are more likely to experience subsequent symptomatic UTI than women who do not have asymptomatic bacteriuria [17].

One of the causes of asymptomatic bacteriuria is the normal flora of the gut, which ascends up the urethra into the bladder and potentially the kidneys. This can happen during such activities as sexual intercourse or wiping towards the urethra after using the toilet [1,7]. Most female students found among a University population are sexually active as such poor hygiene can lead to bacteria colonization of the urethra [8].

In the past studies focused more on ASB in pregnant women due to the associated risk [14,18-22] hence, it is important to assess the colonization of the urinary tract by bacteria among non-pregnant women.

2. MATERIALS AND METHODS

2.1 Study Area

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

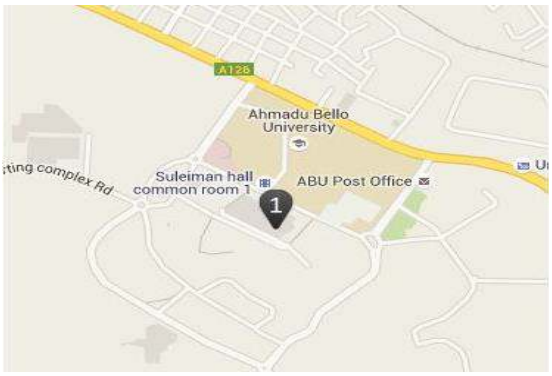

Fig. 1. Google map of Ahmadu Bello University, Zaria

2.2 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.

2.3 Microscopy and Determination of Significant Bacteriuria

A presumptive test method as described by Vandepitte et al. [23] 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).

2.4 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 [24]. A calibrated metal loop was used to transfer 0.001 ml 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℃. The approximate number of bacteria per ml of urine was estimated as described by Chessbrough [24]. 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 ≥ 10^5 colony forming units (CFU) per mL are observed (Stamm and Hooton, 1993) [24] 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 [25,26]. The bacterial isolates were identified using Microgen^{TM} GNA-ID kit following the manufacturer's instructions. A pure 18-24 hour culture of the bacterial isolate to be identified was used. A single colony was emulsified from an 18-24 hour culture in 3mL sterile 0.85% saline for the GN A micro-well test strip and mixed thoroughly. Using a sterile Pasteur's pipette, 3-4 drops (approximately 100 µL) of the bacterial suspension was added to each well of the micro-well test strip(s). After inoculation, wells 1,2 3 and 9 (GN A micro-well test strip counting from the tabbed end) were overlaid with 3-4 drops of mineral oil**.** The top of the micro-well test strip(s) were sealed with the adhesive tape removed earlier and incubated at 35-37℃. The GN A micro-well test strips were read after 18-24 hours incubation. All positive reactions were read with the aid of the color chart and the results were recorded on 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.

2.5 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 [24] and CLSI [27]. Antibiotics tested were obtained from Oxoid Ltd and include; Pefloxacin (5 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Amoxicillin-clavulanic acid (30 µg), Nitrofurantoin

(300 µg), Trimethoprim/Sulfamethoxazole (30 µg), Gentamicin (10 µg), Tetracycline (30 µg) and Ceftriaxone (30 µg). 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 x 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℃ 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 [27], 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 [28].

3. 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.

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

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

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.

Table 2. 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

Table 3. Distribution of isolates

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

The asymptomatic bacteriuria isolates showed high resistance to Tetracycline and Ceftriaxone. Gentamicin, Ciprofloxacin, Pefloxacin, Levefloxcin and Norfloxacin had great antimicrobial activity against the isloates as shown in Table 5.

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.

4. 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 [11], who recorded an incidence rate of 10% of asymptomatic bacteriuria among female students in Lagos state University, Nigeria. Ettehad et al. [29] 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 [30], 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 [5].

This value is higher than 5% incidence bacteriuria among students of secondary schools [31] and females of 14-17 year of age with 3.3% bacteriuria [32]. 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 [8].

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 [33-36]. *Serratia* species, *Pseudomonas aeruginosa* and *Acinetobacter* species are also implicated in asymptomatic bacteriuria [37,38]. 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 [11]. 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 [11].

Table 4. Isolates obtained using microgen identification kit (Microgen^{TM} GNA-ID) to specie level

Isolates	Number (%)
Acinetobacter baumannii	20 (13.51)
Klebsiella oxytoca	17 (11.49)
Escherichia coli	17 (11.49)
Serratia marcescens	12 (8.12)
Klebsiella pnuemoniae	11 (7.43)
Enterobacter agglomerans	11 (7.43)
Salmonella arizonae	10 (6.76)
Enterobacter gergoviae	9 (6.08)
Acinetobacter lwoffi	9 (6.08)
Proteus mirabilis	6 (4.05)
Enterobacter sakazaki	3 (2.03)
Enterobacter aerogenes	3 (2.03)
Yersinia enterocolitica	3 (2.03)
Citrobacter diversus	3 (2.03)
Citrobacter freundii	3 (2.03)
Proteus vulgaris	2 (1.35)
Providencia stuartii	2 (1.35)
Salmonella paratyphi	1 (0.68)
Salmonella choleraesuis	1 (0.68)
Salmonella spp	1 (0.68)
Salmonella gallinarum	1 (0.68)
Klebsiella ozaenae	1 (0.68)
Serratia liquefaciens	1 (0.68)
Shigella serogroup A, B & C	1 (0.68)
Total	148 (100)

Table 5. Resistance profile of bacteria isolates to antibiotics

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

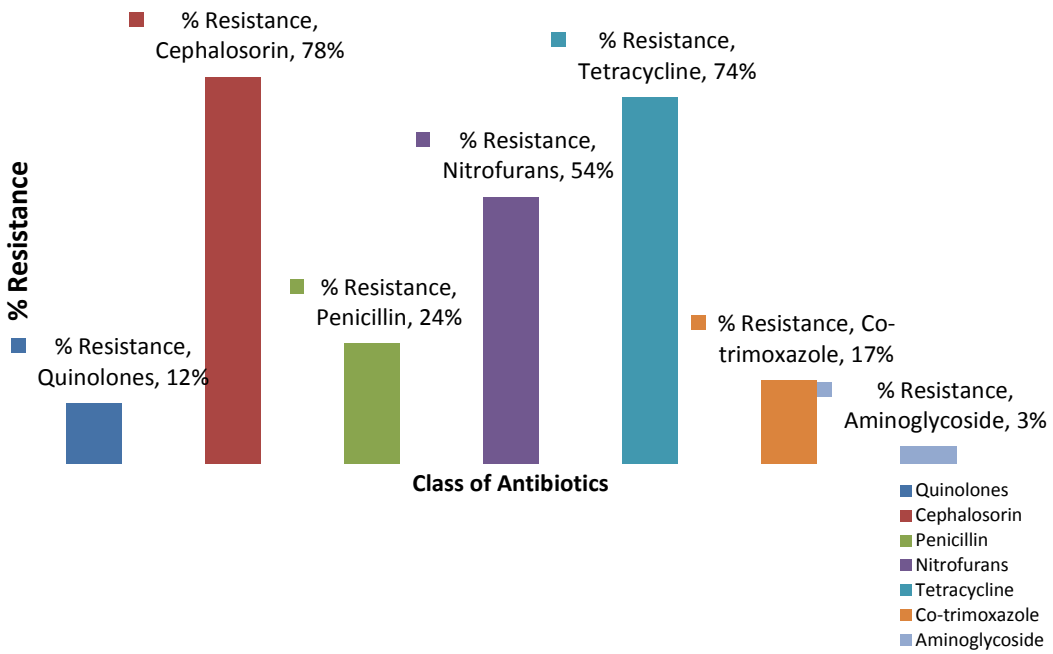

Fig. 2. Resistance of asymptomatic bacteriuria isolates to antibiotics classes

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, [19,39-41] recent reports have shown that organisms such as *Staphylococcus* species, *Klebiella* specie and *Proteus* species are taking the position of *Escherichia coli* [42-44]. The result obtained agrees with the report of Okon et al. [45], 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. [46] 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 [47]. *Acinetobacter* species has been reported to cause urinary tract infection [47], 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. [48] in Bayelsa State, Nigeria and Mansour et al. [49] 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 [50]. 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. [45] and in Benin, Nigeria by Akerele et al. [18]. They reported less resistance to the quinolone group of antibiotics.

5. 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.

6. 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.

CONSENT

We hereby declared that written informed consent was obtained from the patient for publication of this paper and accompanying images.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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