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Asymptomatic Bacteriuria among Apparently Healthy Undergraduate Students in Uyo, South-South, Nigeria

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Authors' contributions

This research work was carried out in collaboration between the two authors. Author OJA designed the work, while author ODA assisted in the collection of samples, identification of the isolates and antibiotic susceptibility testing. Authors OJA and ODA read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: This study was designed to determine the prevalence of asymptomatic bacteriuria among apparently healthy undergraduate students in Uyo, South-South, Nigeria.
Methodology: Microscopic examination, bacteriological analysis and antibiotic susceptibility profile of the bacterial isolates from mid stream urine samples of apparently healthy undergraduate students were carried out using standard microbiological and disc diffusion methods. The presence of glucose, protein, ketone and nitrite were determined using dip sticks.
Results: Microscopical analysis showed that aged 21-25yrs had the highest number of epithelial cells; while the highest yeast cells 6 (21.4%) were obtained in aged 26-30yrs. Only 30 (26.8%) samples had pus cells, 10 (8.9%) crystal cells, 22 (21.4%) cast cells and 26 (23.2%) red blood cells. Of the 112 samples collected, 14 (12.5%), 9 (8.0%), 6 (5.4%) and 4 (3.6%) had glucose, protein, ketone and nitrite, respectively. Age ranged ≤ 20yrs and 21-25yrs had no ketone and nitrite, while highest occurrence of protein was obtained in aged 26-30yrs. Significant bacteriuria (SBU) ranged from 16 (28.6%) in males to 20 (35.7%) in females with highest SBU in age ranged 26-30yrs. The bacteria isolated were *Escherichia coli* 32 (25.0%), *Staphylococcus aureus* 26 (20.3%), *Pseudomonas*

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aeruginosa 16 (12.5%), coagulase negative *Staphylococcus* sp 16 (12.5%), *Streptococcus pyogenes* 10 (7.8%), *Serratia marcescens* 10 (7.8%), *Enterobacter* sp 8 (6.3%), *Klebsiella* sp 6 (4.7%) and *Enterococcus faecalis* 4 (3.1%). Only 86 (67.2%), 90 (70.3%), 64 (50.0%) and 58 (45.3%) of the isolates were sensitive to Streptomycin, Ceftriaxone, Penicillin and Cotrimoxazole, respectively, while 20 (62.5%) of *E. coli* were sensitive to Streptomycin and Ceftazidime. *S. aureus, S. marcescens, Enterobacter* sp and *E. faecalis* were highly sensitive to Ciprofloxacin.

Conclusion: inclusion of assessments of asymptomatic bacteriuria as parts of the medical examination for students' especially new entrants, in tertiary institutions should be adopted.

Keywords: Asymptomatic; bacteriuria; antibiotics; susceptibility; microscopic; Uyo.

1. INTRODUCTION

Asymptomatic bacteriuria is a significant bacterial count in the mid stream urine, usually 10^5 cfu / ml in an individual without any apparent symptoms of urinary tract infections [1]. The importance of asymptomatic bacteriuria lies in the insight it provides into symptomatic infections [2]. The urinary tract consists of various organs of the body involved in the production, storage and excretion of urine [3,4,5]. Urinary tract infection (UTI) is a clinical (symptomatic) or subclinical (asymptomatic) infection caused by pathogenic microbes that invade the entire tract or restricted to either lower tract or upper tract resulting in urethritis, prostitis, cystitis and pyelonephritis [6,7]. Urinary tract infection is one of the most common causes of hospitalization and referral to outpatient, having an estimated figure of 150 million per annum worldwide [8,9,10]. The leading causes of UTI have been reported to be due to *Escherichia coli, Staphylococcus aureus, Proteus* spp*., Klebsiella* spp and *Pseudomonas aeruginosa* [11,12,13]. The relative frequency of the pathogens varies, depending upon age, sex, catheterization, and hospitalization.

The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection, and the patient's ability to mount an immune response to it [5]. Variations exist in the incidence of bacteriuria and subsequent UTI in different countries and this has been attributed to differences in definition, methods of screening and associated risk factors such as age, parity and pregnancy. Early detection of asymptomatic bacteriuria might result in the detection of correctable abnormalities of the urinary tract, reduce the rate of bacteriuria and prevent renal scarring, obstructive atrophy, hypertension, and renal insufficiency [1]. The prevalence and incidence of urinary tract infection or significant bacteriuria is higher in women than in men, which is likely the results of several clinical factors including hormonal effects, behaviour patterns or their having a short urethra and vaginal vestibule which can easily be contaminated [14]. Urinalysis is an essential chemical, physical and microscopic examination of a patient's urine and the results obtained give a valuable and useful picture of the state of kidneys and the urinary tract. The routine chemical testing for pH, protein, glucose, ketones, occult blood, bilirubin, urobilinogen and nitrite are useful parameters in the diagnosis of metabolic and systemic disorders. The most commonly used tests for detecting bacteriuria in asymptomatic persons are dipstick urinalysis and direct microscopy. The dipstick test is rapid, inexpensive, and requires little technical expertise [15]. The prevalence of antimicrobial resistance among urinary pathogens has been on the increase worldwide and the resistance rates to the most commonly prescribed drugs used in the treatment of UTIs vary considerably in different

areas world-wide. The estimation of local etiology and antibiotic susceptibility profile could provide the most effective empirical treatment. Thus, the aim of this study was to screen for presence of glucose, proteins, nitrite, ketone, pus cell, epithelial cell, red blood cell, yeast cell, crystal cells, cast cells and bacterial isolates in the mid stream urine samples from apparently healthy undergraduate students and evaluate the in-vitro susceptibility profile of the isolates to commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Study Area

Uyo is a city in South-Southern Nigeria and is the capital of Akwa Ibom State. Akwa Ibom State shares boundaries with Abia, Cross River and Rivers States. The population in Uyo is estimated to be about 451,128. Uyo is located between latitudes 5º 02' 37" North and longitudes 7º 54' 06" East. Uyo has a Teaching Hospital that serves as a referral centre to both private and public health institutions around.

2.2 Sterilization of Glass wares

All the glass wares used for the research work were thoroughly washed with detergent and rinsed with clean water. The glass wares such as test tubes, Petri dishes, beakers, conical flasks, pipettes, Durham's tubes and McCartney bottles were sterilized using the hot air oven (Model DHG) at 180ºC for one and half hours. Wire loop was heat flamed to redness before and after use.

2.3 Collection of Samples

One hundred and twelve (112) early morning mid-stream urine samples were aseptically collected using sterile, wide-necked, leak-proof universal bottles from apparently healthy undergraduate students (aged ≤ 20 yrs to ≥ 31 yrs) of University of Uyo from July to November, 2012 after giving their informed consent. The sex, age and as well as history of urinary tract infection (UTI) were obtained from the subjects before the collection of samples. Samples were collected from the subjects who were not on antimicrobial therapy at the time of sample collection or who had not taken antimicrobials within 2 weeks prior to sampling and were taken to the Microbiology Laboratory for microbiological analyses.

2.4 Presumptive Screening of Urine for Presence of Glucose, Proteins, Nitrite and Ketone

The presence of glucose, proteins, nitrite and ketone in the mid stream urine samples of apparently healthy undergraduate students were detected using commercially available dip sticks.

2.5 Microscopic Examination of Mid Stream Urine Samples

Two loopful of uniformly mixed uncentrifuged urine samples were aseptically placed on a clean grease-free slide and covered with a cover slip**.** It was examined microscopically to detect the presence of pus cell, epithelial cell, red blood cell, yeast cell, crystal cells and cast

cells using 10x and 40x objectives with condenser iris closed sufficiently to give good contrast.

2.6 Isolation and Identification of Uropathogens

One micro litre (µl) of uncentrifuged, uniformly mixed, mid stream urine (MSU) samples were aseptically inoculated onto Cysteine Lactose Electrolyte Deficient (CLED), MacConkey Agar (MCA) and Blood Agar (BA) media and incubated at 37ºC aerobically for 24 hrs. After incubation the cultures developed on media were observed and the colonies were counted by colony counter. Colony counts of ≥ 10^5 per ml were considered as significant growth. Standard identification procedures of colony morphology, Gram staining reaction, motility, catalase test, oxidase test, urease test, coagulase test, sugar fermentation, indole production and IMViC (Indole, Methyl red, Vogues-Proskauer, and Citrate) tests were used to determine the uropathogens present in the urine samples.

2.7 Antibiotics Susceptibility Test

In vitro susceptibility of the bacterial isolates to antibiotic was determined using disc diffusion technique. Zero point one (0.1) ml of each bacterial isolates prepared directly from an overnight agar plate adjusted to 0.5 McFarland Standard (NCCLS, 2004) was inoculated using sterile pipette onto each of the Petri dishes containing Mueller-Hinton Agar (MHA). The commercially available disc containing the following antibiotics:- Penicillin (Pen, 10ug), Ceftazidime, (Caz,30ug), Streptomycin (Stp,30ug) Ciprofloxacin (Cpf,5ug), Gentamycin (Gen, 10ug), Ofloxacin (Ofl,5ug) Ceftriaxone (Cef,30ug) and Cotrimoxazole (Cot, 30ug) (Oxoid, UK) were aseptically placed on the surfaces of the sensitivity agar plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37ºC for 24 hrs and the zones of inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data. The choice of the above antibiotics used was based on the availability in most of the pharmaceutical shops in the locality. Percentage (%) resistance was calculated using the formula PR = a / b x 100. "PR" is % resistance, 'a' is the number of resistant isolates and 'b' is the number of isolates tested with the antibiotics. The percentage (%) sensitivity was calculated using the formula PS = c/d x 100. "PS" is % sensitivity, 'c' is the number of sensitive isolates and'd' is the number of isolates tested with the antibiotics.

3. RESULTS AND DISCUSSION

The results of the microscopical analysis of the mid-stream urine samples showed that aged 21-25yrs had the highest number of epithelial cells; while the highest yeast cells 6 (21.4%) were obtained in aged 26-30yrs. The results showed that no yeast cells were obtained in age group ≤ 20yrs (Table 1). Of the 112 samples collected, 26 (23.2%), 30 (26.8%), 12 (10.7%), 10 (8.9%), 22 (21.4%) and 26 (23.2%) had epithelial cells, pus cells, yeast cells, crystal, cast cells and red blood cells, respectively (Table 1). The screening of mid-stream urine samples for presence of glucose, proteins, nitrite and ketone using dip sticks showed that the subjects with age ranged ≤ 20yrs and 21-25yrs had no ketone and nitrite. Highest occurrence of protein was obtained in aged 26-30yrs, while 6 (21.4%) of the subjects with age ranged 26-30yrs had glucose in their mid stream urine samples (Table 2). Only 14 (12.5%), 9 (8.0%), 6 (5.4%) and 4 (3.6%) of the samples had glucose, protein, ketone and

Table 1. Microscopy of the urine samples according to age distribution

Age (Yrs)	No of samples collected	Epithelial cells no.(%)	Pus cells no.(%)	Yeast cells no.(%)	Crystal no.(%)	Cast cells no.(%)	Red blood cells no.(%)
≤ 20	28	4(14.3)	4(14.3)	0(0.0)	2(7.1)	4(14.3)	4(14.3)
21-25	28	10(35.7)	6(21.4)	4(14.3)	2(7.1)	8(28.6)	8(28.6)
26-30	28	8(28.6)	12(42.9)	6(21.4)	4(14.3)	8(28.6)	6(21.4)
≥ 31	28	4(14.3)	8(28.6)	2(7.1)	2(7.1)	2(7.1)	8(28.6)
Total	112	26(23.2)	30(26.8)	12(10.7)	10(8.9)	22(21.4)	26(23.2)

nitrite, respectively (Table 2). The percentage occurrences of significant bacteriuria (SBU) among the subjects are shown in Table 3. The significant bacteriuria (SBU) among the females are 2 (14.3%), 6 (42.9%), 8 (57.1%) and 8 (57.1) in age ranged ≤ 20yrs, 21-25yrs, 26-30yrs and ≥ 31, respectively while the corresponding values obtained in males are 2 (14.3%), 4 (28.6%), 6 (42.9%) and 4 (28.6) in age ranged ≤ 20yrs, 21-25yrs, 26-30yrs and ≥ 31, respectively. (Table 3)

Table 2. Presumptive screening of urine for presence of glucose, proteins, nitrite and ketone

Age (Yrs)	No of samples collected	Glucose no. (%)	Protein No. (%)	Ketone no. (%)	Nitrite no. (%)
≤ 20	28	0 (0.0)	2 (7.1)	0 (0.0)	0 (0.0)
21-25	28	2 (7.1)	2 (7.1)	0 (0.0)	0 (0.0)
26-30	28	6 (21.4)	4 (14.3)	2 (7.1)	2 (7.1)
≥ 31	28	6 (21.4)	1 (3.6)	4 (14.3)	2 (7.1)
Total	112	14 (12.5)	9 (8.0)	6 (5.4)	4 (3.6)

Table 3. The percentage occurrence of significant bacteriuria (SBU) among undergraduate students

Age (Yrs)	No of samples collected	Significant bacteriuria female no.(%)	No of samples collected	Significant bacteriuria male no.(%)
≤ 20	14	2 (14.3)	14	2 (14.3)
21-25	14	6 (42.9)	14	4 (28.6)
26-30	14	8 (57.1)	14	6 (42.9)
≥ 31	14	6 (42.9)	14	4 (28.6)
Total	56	20 (35.7)	56	16 (28.6)

The results of the morphological and biochemical characteristics of both the Gram positive and Gram negative bacterial isolates from the urine samples are shown in Table 4. One hundred and twenty-eight (128) bacterial isolates were obtained and the most predominant

Table 4. Morphological and biochemical characteristics of bacteria isolated from mid-stream urine samples

Parameters	a	b	c	d	e	F	g	h	i
Grams reaction	+/cocci	+/cocci	+/cocci	-/rod	-/rod	+/cocci	-/rod	-r/rod	-/rod
Catalase test	-	+	+	-	-	-	-	-	-
Citrate test	-	-	-	-	-	-	+	+	-
Coagulase test	-	+	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	-	+
Indole test	-	-	-	+	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	+
Urease activity	-	-	-	-	-	-	-	+	-
Glucose	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	+	+	-	-	+	-
Sucrose	-	-	-	-	-	-	+	+	-
Mannitol	+	+	+	+	+	+	+	+	+
Vogues Proskauer	-	+	+	-	+	-	+	+	-

Keys: - : negative; + : positive ; a: Streptococcus pyogenes; b: Staphylococcus aureus; c : CON- Staphylococcus sp d: Escherichia coli; e: Enterobacter sp; f: Enterococcus faecalis; g: Serratia marcescens ; h: Klebsiella sp; i: Pseudomonas aeruginosa

bacteria isolated was *Escherichia coli* 32 (25.0%), followed by *Staphylococcus aureus* 26 (20.3%), the frequencies of other bacteria were as follows: *Pseudomonas aeruginosa* and coagulase negative *Staphylococcus* sp 16 (12.5%) *Streptococcus pyogenes* and *Serratia marcescens* 10 (7.8%) each, *Enterobacter* sp 8 (6.3%), *Klebsiella* sp 6 (4.7%) and *Enterococcus faecalis* 2 (3.1%) (Table 5)

In-vitro susceptibility profiles of the bacterial isolates to antibiotics are shown in Figs i-ix. The results showed that of the 128 bacterial isolates obtained, 86 (67.2%), 90 (70.3%), 64 (50.0%) and 58 (45.3%) were sensitive to Streptomycin, Ceftriaxone, Penicillin and Cotrimoxazole, respectively. Only 20 (62.5%) of *E. coli* were sensitive to Streptomycin and Ceftazidime (Fig i). All the *Klebsiella* sp were sensitive to both Ceftriaxone and Ciprofloxacin (Fig ii). *Staphylococcus aureus* were highly sensitive to ciprofloxacin and ceftriaxone (Fig iii), both *Streptococcus pyogenes and Pseudomonas aeruginosa* were highly resistant to cotrimoxazole (Figs iv and v), 8 (50.0%) Coagulase negative *Staphylococcus* sp were resistant to ceftriaxone, penicillin and cotrimoxazole (Fig vi), 10 (100%) S*erratia marcescens* were sensitive to Gentamycin (Fig vii). All *Enterobacter* sp were sensitive to Ciprofloxacin, Ceftazidime and Gentamycin (Fig viii), while all *Enterococcus faecalis* obtained were resistant to Penicillin and Cotrimoxazole (Fig ix).

Table 5. Prevalence of Bacterial Isolates Obtained from Mid-stream Urine Samples

Bacterial isolates	No of occurrences	% of occurrences
Escherichia coli	32	25.0
Klebsiella sp	6	4.7
Staphylococcus aureus	26	20.3
Streptococcus pyogenes	10	7.8
Pseudomonas aeruginosa	16	12.5
CON-*Staphylococcus* sp	16	12.5
Serratia marcescens	10	7.8
Enterobacter sp	8	6.3
Enterococcus faecalis	4	3.1
Total	128	100

Key: CON: Coagulase negative

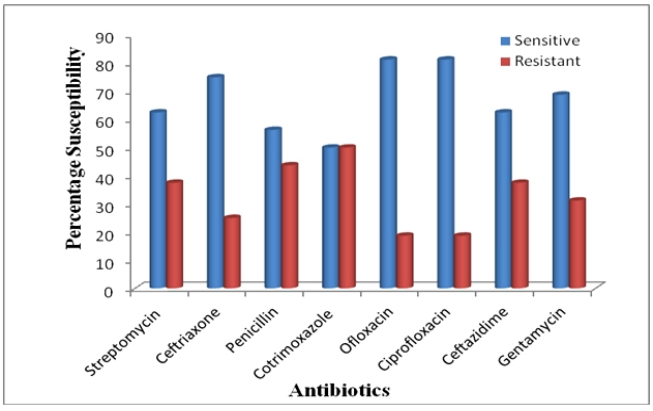

Fig. i. Antibiotic Susceptibility Profile of *E. coli* Isolated from the Mid-stream Urine

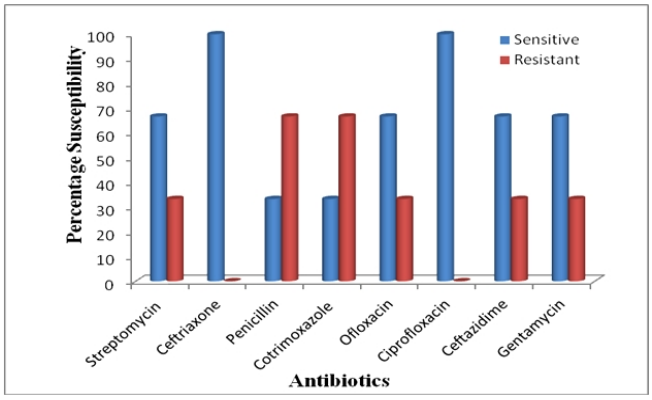

Fig. ii. Antibiotic Susceptibility Profile of *Klebsiella* sp Isolated from the Mid-stream Urine

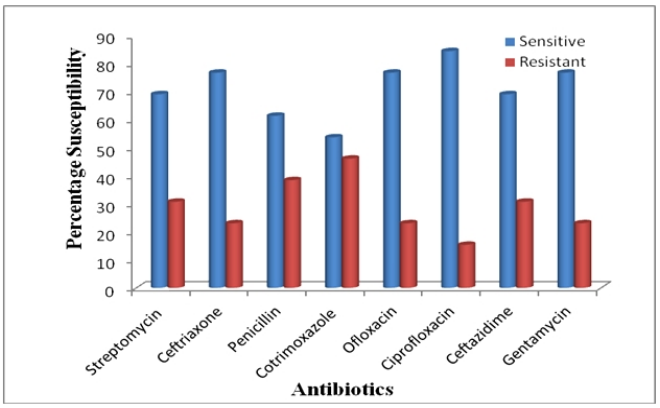

Fig. iii. Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolated from the Mid-stream Urine

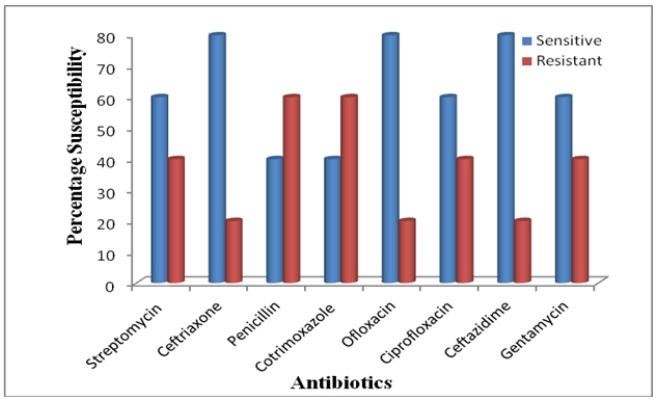

Fig. iv. Antibiotic Susceptibility Profile of *Streptococcus pyogenes* Isolated from the Mid-stream Urine

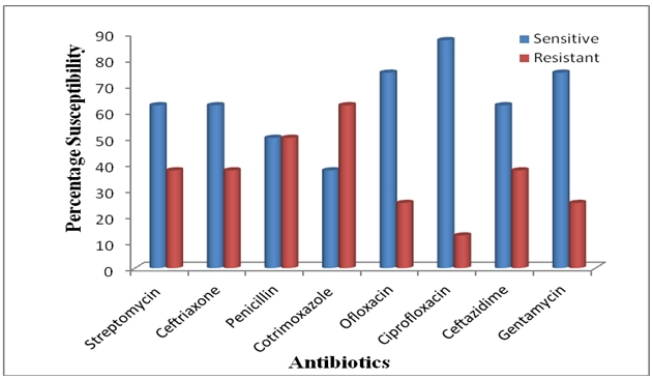

Fig. v. Antibiotic Susceptibility Profile of *Pseudomonas aeruginosa* Isolated from the Mid-stream Urine

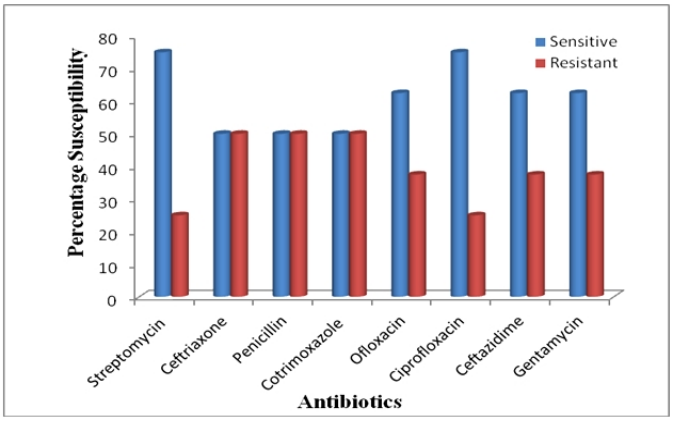

Fig. vi. Antibiotic Susceptibility Profile of Coagulase negative *Staphylococcus* sp Isolated from the Mid-stream Urine

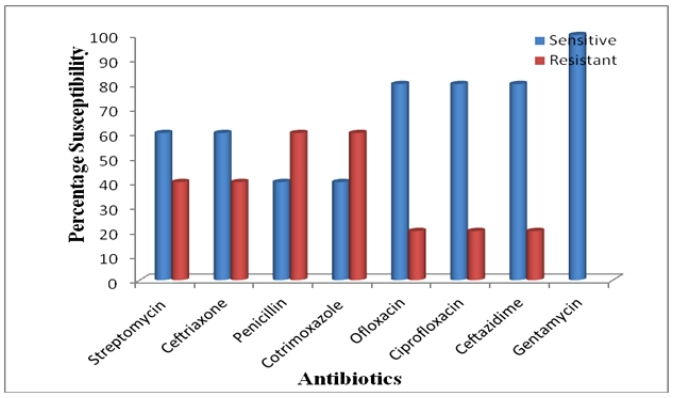

Fig. vii. Antibiotic Susceptibility Profile of Se*rratia marcescens* isolated from the Mid-stream Urine

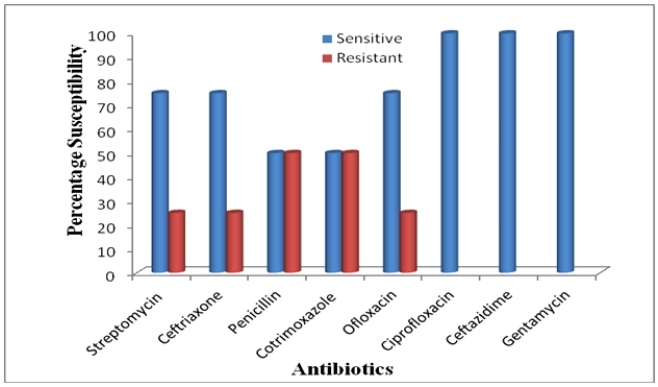

Fig. viii. Antibiotic Susceptibility Profile of *Enterobacter* sp *i*solated from the Mid-stream Urine

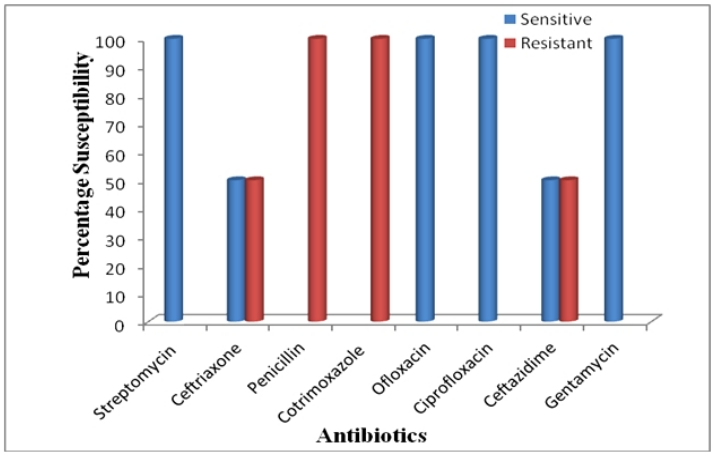

Fig. ix. Antibiotic Susceptibility Profile of *Enterococcus faecalis* isolated from the Mid-stream Urine

Mid stream urine samples are among the most numerous of specimens sent for microbiological analysis in order to reduce the morbidity and mortality caused by urinary tract infections [5]. Urine culture is regarded as the gold standard for screening of asymptomatic bacteriuria among population**.** Microscopic examinations of mid-stream urine samples of undergraduate students revealed the presence of epithelial cells, crystal cells, pus cells, cast cells, yeast cells, and red blood cells. The presence of pus cell in mid-stream urine samples has been recorded by [16]. The urinalysis in this study showed that 4 (3.6%) samples were positive to nitrite and this is in conformity with [17]. The presence of nitrite has a predictive value for urinary tract infection, since nitrite is formed as a metabolic product from bacteria that breakdown nitrate to nitrite. The proportion of students with proteinuria in this study was 8.03% and this was higher than the values obtained in Northern Iran (1.6%), Tokyo (0.08%) and Egypt (0.12%) [18].The presence of significant bacteriuria among the students in this study agrees with previous findings of [19,20]. They showed the presence of bacteria in urine without the occurrence of physical symptoms of urinary tract infection. The highest significant bacteriuria (SBU) was found among subjects aged 26-30 yrs and this is in contrary to the findings of [21], whose highest SBU was reported in the aged 35 to 39 yrs.

Several studies have demonstrated the geographical variability of pathogens occurrence in cases of urinary tract infection / significant bacteurria among populations with the predominance of Gram-negative bacteria especially Enterobacteriaceae such as *E. coli* and *Enterobacter* spp, while in some regions of the world, *S. aureus* showed the highest prevalence of bacterial pathogen, followed by *E. coli.* The isolation of *E. coli, Enterococcus* sp., *Klebsiella* sp., *Proteus mirabilis.*, *P. aeruginosa* and *S. aureus* from the mid steam urine samples is in agreement with [22], who reported the prevalence of *E. coli, Enterococcus* sp., *Klebsiella* sp., *Proteus* sp., *P. aeruginosa* and *S. aureus* in the urine samples. *E. coli* had the highest prevalence in this study and this is similar to the reports of [22], but differs from the reports of [23]. Results from several studies have shown that the proportion of *E. coli* as a principal causative agent of UTIs is slowly declining, being replaced by other members of the Enterobacteriaceae and *Enterococci.* Although, *E. coli* was the most common uro-pathogen in this study, there is a difference in its prevalence rates when compared with

other reports, which gave a higher prevalence rate of 60 - 90% of *E.* coli than other isolates [24].
Female had the higher occurrence of asymptomatic bacteriuria than the male in this study and the higher occurrence of urinary tract infections / asymptomatic bacteriuria in females might be as a result of a variety of factors, such as the close proximity of the female urethral meatus to the anus (female anatomic feature contributes to higher prevalence), in-coordinate voiding of urine in female is often associated with constipation and encourages infection of the urinary tract (significant bacteurria). Also, vaginal microflora also play a critical role in encouraging vaginal colonization with coliforms and this can lead to urinary tract infection.

The results of the antibiotic susceptibility profiles of the bacterial isolates from mid stream urine samples of the subjects with asymptomatic bacteriuria showed varied percentages of sensitivity and resistance. The high sensitivity of *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* to ciprofloxacin in this study is similar to the results of [25,26], but the finding contradicts the reports of [27], where *E. coli* was reported to be resistant to ciprofloxacin. The high sensitivity of the bacterial isolates to gentamycin and ofloxacin confirms the previous reports of [28]. Gentamycin is administered parenterally and, therefore, due to the discomfort of injection, it is less likely to be misused than oral drugs [29]. The resistant of *Staphylococcus aureus* and *E. coli* to streptomycin in this study is in agreement with the results obtained by [25]. [30] reported the resistance of the uro-pathogens to some cephalosporins and this confirms the results obtained in this study. The observed resistance to the antibiotics is a probable indication of earlier exposure of the isolates to these drugs and / or indiscriminate use of antibiotics among the undergraduate students, which has favoured the emergence of resistance strains. Inappropriate use of various classes of antimicrobials may lead to subclinical infections including UTIs, but more importantly, may create an avenue for the development of resistant organisms in the community.

4. CONCLUSION

In conclusion, early detection of asymptomatic bacteriuria among the students may reduce the rate of bacteriuria and prevent symptomatic infection and its complications. Also, inclusion of assessments of asymptomatic bacteriuria as parts of the medical examination for students' especially new entrants, in tertiary institutions should be adopted.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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