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Studies on Symptomatic Urinary Tract Infection and Asymptomatic Bacteriuria in a University Community

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The research was to investigate the bacteria associated with symptomatic (urinary tracts infection) and asymptomatic bacteriuria in the University of Uyo, Nigeria. A total of 100 mid-stream urine samples were collected and analyzed. Seventy-six percentage of the total urine samples screened were found to have significant bacteriuria, out of which a total of 120 isolates were obtained while 24% of the samples analysed showed no significant bacteriuria. Bacterial proximate composition revealed the presence of *Escherichia coli* (41. 7%), *Staphylococcus aureus* (16.7%), *Klebsiella* spp. (8.3%), *Streptococcus faecalis* (6.7%), *Proteus* spp. (6.7%), *Pseudomonas* spp (6.7%), *Aerobacter aerogenes* (6.7%), *S. saprophyticus* (5.0%), and *Nocardia* spp. (1.7%). These isolates principally constituted the total heterotrophic bacterial counts which ranged from 2.4 × 10^6cfu/ml to 15.8 × 10^6cfu/ml observed in the urine samples. Samples obtained from volunteers with age range 21 – 25years had the highest percentage (80.8%) of symptomatic bacteriuria and 15 – 20years had the least percentage of infection. More female individuals were affected than male ($p < 0.05$) with a total of 78 (65.0%) of the isolates from female individuals. Of these, 36 (30.0%) were identified as *Escherichia coli*. The Antibiotic profile showed that between 18.8% and 34.4% of the isolates were susceptible to gentamycin, nalidixic acid, erythromycin and nitrofurantoin; 28.6% showed intermediate profile to erythromycin while 92.9% to 100% of the isolates were resistant to tetracycline, colistin, cotrimoxazole, cloxacillin, penicillin, ampicillin and chloramphenicol. A range of 16.7 % to 50% multidrug resistant and beta lactamase producing isolates were cured of their plasmids and became susceptible to tetracycline, penicillin and ampicillin. The presence of higher significant asymptomatic bacteriuria might be a result of unhygienic living and sexual habit.

Key words: Symptomatic, Bacteriuria, Antibiotic resistance, Plasmids, Acridine orange.

Asymptomatic bacteriuria implies the presence of significant bacterial count of 10^5 cfu/ml in the urine of an individual without symptoms[1]. When this condition is accompanied with symptom (s) or syndrome, it becomes symptomatic Urinary Tract Infection (UTI)[2]. It has also been ascribed as the most common bacterial infection in older populations, both in the community and in the nursing home[3]. Salem *et al.*[4] also reported the incidence of bacteriuria among 30% of Egyptian children and adolescent with diabetes. A higher incidence of significant bacteriuria of 10% in University student than 5% in secondary school student was observed by Olaitan[5] which she attributed to increase in sexual activities among the university age group. The frequently isolated bacteria in both symptomatic and asymptomatic bacteriuria include *Escherichia coli, Klebsiella pneumonia, Enterococcus fecalis, Pseudomonas aeruginosa* etc[5,6].

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Symptomatic urinary tract infections are divided into either Lower Urinary Tract Infection (LUTI) which includes acute cystitis or Upper Urinary Tract Infection (UUTI) such as acute pyelonephritis[7,8] The individuals with symptomatic UTI, unlike asymptomatic may experience symptoms like sudden rise in micturition frequency and dysuria, burning pain in the urethra during urination, excruciating pubic pain in women and sense of pressure or pain in men's rectum during and after voiding. There may also be an unpleasant odour, cloudiness of urine and gross hematuria[9,10]. The progression of the infection may extend to kidney and uterus causing back pains, chills, fever, nausea and vomiting[11].

Large cases of childhood mortality and morbidity have been attributed to infections of urinary tract origin[12,13], even as patients pre-operative UTI have been observed to contribute to postoperative delayed wound healing[14]. About 95% of cases of UTIs are caused by bacteria which invade urethral opening, migrating towards the bladder. So, the infection outcome depends on the promptness of diagnosis and treatment while the therapeutic prognosis will be dictated by effective sensitivity testing prior to antibiotic administration and/or restoration of immune competence in the immunosuppressed[15].

Sero-prevalence of HIV infection in Uyo, Akwa Ibom State, Nigeria was reported to be 34.17%, which made the state ranked 4th in Nigeria[16]. Also, venereal diseases are also rampant in the state[17]. So, this study becomes imperative. The progression of infection in symptomatic bacteriuria to symptomatic urinary tract infection may occur[18,19] and even lead to higher complications[20] especially among the immunocompromised or immunosupressed individuals. The study was designed to provide the information on potential pathogenic bacteria in both asymptomatic and symptomatic bacteriuria cases among the student of University of Uyo as they come from various cities in the state.

METHODS

Choice of Materials

The choice of the antibiotic used in this study was informed by prior study (unpublished) of frequent prescriptions within the study area while the sample populations were representative sampled round the various cities in the state.

Description of the Study Area; Samples Collection and Processing

The study took place at the Department of Microbiology, University of Uyo and the University Medical Center. Midstream urine samples of 10–15ml were obtained from the volunteers and vortex gently without spilling. Exact 5 ml was immediately transferred into double strength thioglycolate broth while the remaining was preserved in ice. Demographic features including occupation, age and sex were collected with the aid of a questionnaire. Samples from thioglycolate incubated overnight at 37ºC were subcultured by streak plate technique while the urine sample under ice were immediately analyzed by pour plate techniques using cysteine lactose electrolyte deficient agar (CLED) (oxoid), MacConkey agar (oxoid), mannitol salt agar (oxoid) and eosin methylene blue agar (oxoid) for viable bacterial counts. Plates were incubated aerobically at 37°C overnight. Colony counts carried out from pour-plates were interpreted as significant using the standards of $\geq 10^5$ cfu/ml. Isolates were identified from streak plates with reagents to species level using conventional methods[21, 23] and kits systems.

Antibiotic Susceptibility Testing (AST)

Inhibitors of the cell wall synthesis (penicillin, ampicillin, augmenting, amoxycillin, cloxacillin and ceftriazone), protein synthesis (gentamycin, streptomycin, tetracyclines, chloramphenicol and erythromycin), and nucleic acid synthesis (ciprofloxacin and cotrimoxazole) etc were utilized for AST. The disc diffusion method for in-vitro antibiotic susceptibility test described by Bauer *et al*.[22] and Cheesebrough[23] were used in this study.

Interpretation and evaluation of the antimicrobial susceptibility

The interpretation of the diameters/zones of inhibition before the test antibiotics was carried out based on the manufacturers' interpretation manuals. The percentage resistance was calculated as follows: Percentage resistance = (No of resistant isolates/No of isolates tested with the antibiotic) × 100.

β-lactamase Test

Based on the resistance of the isolates to

penicillin and other β-lactam antibiotics used, β -lactamase test was carried out using the method of Odugbemi *et al*.[24]. Strips of starch paper about 4 – 7cm were cut and sterilized using 70% ethanol, the strips were soaked for 10 minutes in benzyl penicillin dissolved in phosphate buffer with 100,000 units. The cut strips were then spread evenly on Petri dishes and about 18 – 24 hours old cultures grown on Nutrient Agar were inoculated on the surface of the test paper and spread over an area of 2 – 3mm. The Petri dishes were incubated at 37°C for 30 minutes after which Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turned uniformly black within 30 seconds of application. Colonies with decolourized zones are positive for β-lactamase but colonies with black background showed β -lactamase negative. The result was read within 5 minutes to avoid false positive result.

Plasmid Curing

Plasmid curing to presumptively evaluate the role of plasmid on the observed resistance was carried out according to Silhavy *et al*.[25]. A measure of 0.1mg/ml of acridine orange was supplemented in freshly prepared nutrient broth. The isolates from the stock were inoculated into the broth and incubated for 24 hours. Using the overnight culture, antibiotic sensitivity tests were carried out and allowed for 18 – 24 hours after which observation of clear zones (indicating susceptible) was made in a formerly resistant strain which was indicative of plasmid mediated resistance.

RESULTS

Bacterial Counts and Sample-Isolates Relationships

A total of 120 bacteria isolates were obtained from the 100 samples analyzed. From the biometrics of the patients, 68% were females and 32% were males and none of the individuals with asymptomatic bacteriuria had previous history of urinary tract infection. Out of the 100, 76% were positive for significant bacteriuria made up of 50.0% females and 26% males. In the samples with significant bacterial growth, the total aerobic heterotrophic bacterial count ranged from 2.4×10^6 cfu/ml to 1.6×10^7 cfu/ml, bacterial counts from

Table 1. Frequency of bacteria isolated from urine samples (n=100)

Organisms	*E. c*	*S.a*	*K.* spp.	*S. f*	*Pr.* spp.	*P.* spp.	*A. a.*	*S. s.*	*N.* spp.	Total
% of total bacteria isolated	41.7	16.7	8.3	6.7	6.7	6.7	6.7	5.0	1.7	100

Key: *E.c=E. coli, S.a=S. aureus, K.*spp=*Klebsiella* spp., *S.f=S. faecalis, Pr.* spp.=*Proteus spp*
P. spp.=*Pseudomonas* spp., *A.a=Aerobacter aerogenes, S.s=S. saprophyticus* and *N.* spp.=*Nocardia* spp.

Table 2. Sex-related pattern of asymptomatic bacteriuria among the volunteers

Organisms	Isolates					Overall %
	Total	Male	% Infection	Female	% Infection	Infected Individuals
E. coli	26	6	5.0	20	16.7	21.7
S. aureus	12	8	6.7	4	3.3	10.0
Klebsiella spp	6	2	1.7	4	3.3	5.0
S. faecalis	4	2	1.7	2	1.7	3.3
Proteus spp	6	4	3.3	2	1.7	5.0
Pseudomonas spp	4	0	0.0	4	3.3	3.3
Aerobacter aerogenes	4	2	1.7	2	1.7	3.3
S. saprophyticus	4	2	1.7	2	1.7	3.3
Nocardia spp	2	0	0.0	2	1.7	1.7
Total	68	26	21.7	42	35.0	56.7

asymptomatic bacteriuria ranged from 5.2×10^6 to 1.58×10^7 cfu/ml while those of symptomatic bacteriuria ranged from 2.4×10^6 to 1.57×10^7 cfu/ml (Table 1 and Fig 1).

Mid-stream urine samples proportion per age group are shown in Fig 1. In all, the cultural, morphological and biochemical properties of these isolates confirmed them to belong to the genera Escherichia, Staphylococcus, Klebsiella. Streptococcus, Proteus, Pseudomonas, Aerobacter, and Nocardia. The observed bacterial prevalence among the asymptomatic carriers

Table 3. Sex-related pattern of urinary tract infection among individuals studied

Organisms	Isolates					Overall % Infected
	Male	% Infection	Female	% Infection	Total	Individuals
E. coli	8	6.7	16	13.3	24	20.0
S. aureus	4	3.3	4	3.3	8	6.7
Klebsiella spp	0	0.0	4	3.3	4	3.3
S. faecalis	2	1.7	2	1.7	4	3.3
Proteus spp	0	0.0	2	1.7	2	1.7
Pseudomonas spp	0	0.0	4	3.3	4	3.3
Aerobacter aerogenes	2	1.7	2	1.7	4	3.3
S. saprophyticus	0	0.0	2	1.7	2	1.7
Nocardia spp	0	0.0	0	0.0	0	0.0
Total	16	13.3	36	30.0	52	43.3

Table 4. Antibiotic susceptibility profile in no (%) for Gram negative bacteria

S. No.	Antibiotic codes	Number and percentage (%) of resistant isolate	Number and percentage (%) of intermediate	Number and percentage (%) of sensitive isolate
1	Ampicillin	64 (100.0)	0 (0)	0(0)
2	Cotrimoxazole	60 (93.8)	0 (0)	4(6.3)
3	Gentamycin	32 (50.0)	10 (15.6)	22(34.4)
4	Nalidixic Acid	42 (65.6)	10 (15.6)	12(18.8)
5	Nitrofurantoin	42 (65.6)	10 (15.6)	12 (18.8)
6	Colistin	60 (93.8)	0 (0)	4 (6.3)
7	Streptomycin	40 (62.5)	6 (9.4)	18 (28.1)
8	Tetracycline	54 (84.4)	0 (0)	10 (15.6)

Table 5. Antibiotic susceptibility profile in no (%) for Gram positive bacteria

S. No.	Antibiotic codes	Number and percentage (%) of resistant isolate	Number and percentage (%) of intermediate	Number and percentage (%) of sensitive isolate
1	Ampicillin	56 (100)	0 (0)	0 (0)
2	Chloramphenicol	56 (100)	0 (0)	0 (0)
3	Cloxacillin	56 (100)	0 (0)	0 (0)
4	Erythromycin	28 (50.0)	16 (28.6)	12 (21.4)
5	Gentamycin	44 (78.6)	6 (10.7)	6 (10.7)
6	Penicillin	56 (100)	0 (0)	0 (0)
7	Streptomycin	50 (89.3)	4 (7.1)	2 (3.6)
8	Tetracycline	52 (92.9)	0 (0)	4 (7.1)

showed *Nocardia* spp. (1.7%) and *Escherichia* spp. (41.7%) as least and highest respectively. This was followed by Staphylococcus (13.3%), Klebsiella (5.0%), Streptococcus (3.3%), Proteus (5.0%), Pseudomonas (3.3%) and Aerobacter (3.3%) while for symptomatic (UTI) patients had the genera Escherichia (20.0%), Staphylococcus (8.3%), Klebsiella (3.3%), Streptococcus (3.3 %), Proteus (1.7 %), Pseudomonas (3.3 %) and Aerobacter (3.3) (FIG 2). In Table 1, samples obtained from (21-25 years) have the highest infection rate of 21 (80.8%). We observed from the 20 volunteers (15 – 20 years) tested that 14 (70.0%) had infection. Twenty (71.4%) had bacteriuria from 28 volunteers (26 – above) tested.

The sex-related pattern of urinary tract infection (Tables 2 and 3) among 120 isolates from 100 urine samples revealed that more female students 36 (69.2%) and 42 (61.7%) compared to male students 16 (30.8%) and 26 (38.2%) had urinary tract infection and asymptomatic bacteriuria respectively. The most frequently occurring isolate in female students was *Escherichia coli* (20 [16.7%]) of the total isolates while the most frequently occurring isolates in males was *S. aureus* (8 (6.7%)) of the isolates for both symptomatic and asymptomatic samples collected. In all, *E. coli* was most prevalent in asymptomatic bacteriuria (21.7%) for both male and female individuals.

Antibiotic Profile

All bacterial isolates from significant bacteriuria in urine from some individuals from University of Uyo community exhibited varying susceptibilities to the antimicrobial agents used in this study. It was observed that gentamycin,

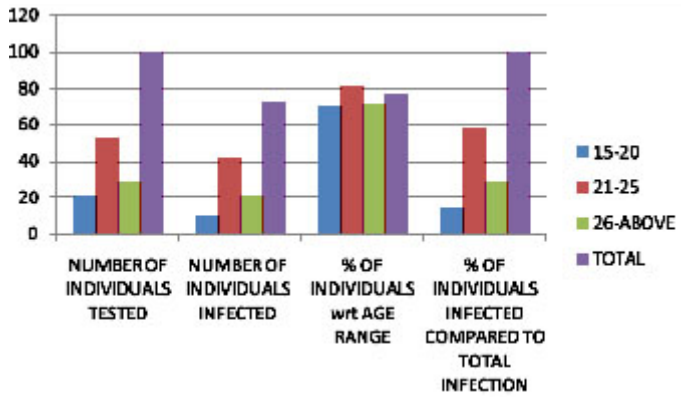

Fig. 1. Age related pattern of bacteriuria infection among individuals (n=100) examined

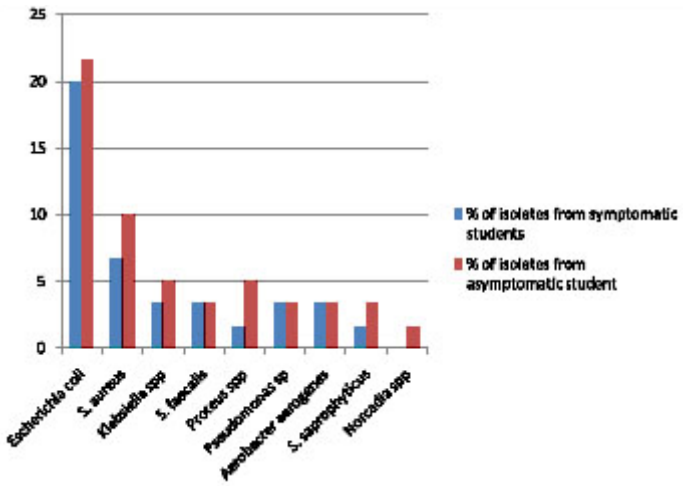

Fig. 2. Comparison of bacteria isolated from symptomatic and asymptomatic urine samples

nalidixic acid, streptomycin, nitrofurantoin and erythromycin exhibited high activity against the Gram positive and Gram negative isolates while colistin, tetracycline and cotrimoxazole were less effective against them (Tables 4 and 5). A varying multiple antibiotic resistant was observed with an index that ranged from 0.19 to 0.78. None of the isolates exhibited 100% susceptibility. Four (12.5%) isolates were resistant to 7 of the antibiotics employed in Gram negative disc while 10(35.7%) of the isolates were resistant to 7 antibiotics (Gram positive disc) employed. Five (15.6%) of the isolates were resistant to 6 antibiotics (Gram negative) employed while 6(21.4%) of the isolates was resistant to 6 antibiotics (Gram positive). One (3.6%) of the isolate was resistant to 5 antibiotics (Gram positive) employed while 6 (18.8%) of the isolates were resistant to 5 antibiotics (Gram negative) employed.

A 50% curing rate was observed in Gram negative bacteria as 50% of the resistant isolates tested became susceptible after curing experiment with acridine orange. However, a curing rate of 16.7% was observed for tetracycline antibiotic in Gram positive *Staphylococcus* spp.

DISCUSSION

The outcome of this study established the prior fear on the pathogen holding proportion in urine of the population from which the study was conducted. An observation of higher prevalence among the female volunteers (35%) than male (21.68%) made in this study was in conformity with earlier observation by other researchers[26-28]. In term of age, a highest prevalence of 58.3% was observed among volunteers of age range 21-25 years followed by 27.8% among volunteers of age range (15-20) years. These age groups have been noted as active sex group, to which highest prevalence of venereal diseases have frequently been attributed[29-31].

Barrett[32]; Kahlmeter[33] and Olaitan[5] reported that *Escherichia coli* is usually the most frequently implicated in urogenital samples assessment (especially in female individuals). In this study, similar trend was observed as *Escherichia coli* occurrence was 41.67% followed by *S. aureus*. Other isolates followed that similar trend in both symptomatic and asymptomatic cases, but *Norcadia* spp. (1.67%) was the least as it was only found in few cases of asymptomatic bacteriuria only. Meanwhile, the observation of *Norcadia* spp. in the urine of the individuals is suggestive that they are immunosuppressed or immunocompromised[34]. If immunosuppressed, this may be through drug's contra-indication just as the individual might be a retroviral patient. This might also be an indication of poor hygiene among the individuals as *Norcadia* spp. are common natural inhabitants of the soil throughout the world. The individuals involved may exhibit subcutaneous abscesses due to complication arising from the presence of Norcadia *in vivo*[35-37]. More of the *Escherichia coli, S. aureus, Klebsiella* spp. *S. faecalis* and *Proteus* spp. were recovered from asymptomatic bacteriuria cases than symptomatic urinary tract infection and may be due to poor sanitation or hygiene. The reduction in microbial load and diversity in symptomatic bacteriuria compared to asymptomatic might also be as a result of prior self medication by individuals with symptomatic urinary tract infection, due to unpleasant symptom they suffered before reporting at the medical centre.

It was observed that gentamycin, nalidixic acid, streptomycin, nitrofurantoin and erythromycin exhibited high activity against the Gram positive and Gram negative isolates while colistin, tetracycline and cotrimoxazole were less effective against them (Tables 4 and 5). This observation did not agree with the observation of Olaitan[5] who observed co-trimoxazole as the most effective antibiotic. Our observation on nitrofurantoin and some β- lactam antibiotics however are in line with report of Barrett *et al*.[32]. A varying multiple antibiotic resistant was observed with indices that ranged from 0.19 to 0.78. This denotes concomitant resistance to many antibiotics by each of the isolates. More importantly, suggest high-risk sources where antibiotics are in abuse leading to high selective pressure.

The role of plasmid in the observed resistance to the β- lactam antibiotics employed in this study came to light as 50% of the isolates turned sensitive after curing with acridine. The acridine orange inhibits the plasmid replication causing the previously resistant isolates to become susceptible[37].

The study population that have been

estimated to have over 30% chance of immunocompromised individuals[16] needs to be protected from the pathogens observed in both symptomatic and asymptomatic cases. This is because they stand a great risk of opportunistic infection in a devastating proportion. To achieve this, sanitary prophylaxis is preferentially recommended, as medical prophylaxis may predicate more devastating antibiotic resistance[38]. Given a condition of immunosuppression of any sort, the commensals on the unkempt immunocompromised individuals utilize the opportunity to exhibit their pathogenic potentials[39]. Adequate intake of fluid is recommended to make sufficient water available for flushing out the bacteria in urogenital pathway by mechanical barrier of non specific immunity[40]. This will go a long way in preventing the establishment of pathogen and hinder the progression of asymptomatic bacteriuria to symptomatic urinary tract infection.

When necessary, however, antibiotic therapy should be used, but antibiotic susceptibility test should precede the choice of best and cost effective antibiotic. Self medication in this regards should be discouraged. Public enlightenment should be intensified to reduce the indecent sexual behavior among young individuals regarded as active sex group. By and large, this will prevent the spread of pathogen in epidemic proportion that may be difficult to control.

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