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Isolation of Urinary Tract Infection Organisms from Apparently Healthy Student of University of Benin

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Abstract

Urinary tract infections (UTIs) are the most common infections affecting people worldwide. Urinary tract infections are caused by both Gram-negative and Gram-positive bacteria, as well as certain fungi. The aim of the study is to isolate urinary tract infection bacteria from apparently healthy students of University of Benin. A total of one hundred and fifty (150) urine samples were collected and screened using cultural and biochemical methods and multiple antibiotic susceptibility was evaluated using Kirby Bauer disc diffusion technique. Organisms isolated from the urine samples were Staphylococcus aureus, Escherichia coli, Proteus mirabilis and Klebsiella spp. The result revealed that female had the highest percentage occurrence of Escherichia coli (42.54%), Staphylococcus aureus (34.25%), Proteus spp (12.71%), while male had the highest occurrence of Klebsiella spp (39.02%). It was observed that most of the isolates where resistant to Amoxicillin, Ciprofloxacin, Cefixime and Cefuroxime but susceptible to Ofloxacin, Augmentin and Gentamicin. All the isolates had multiple antibiotic resistant index of >0.2. The increase in resistance of the isolates to the antibiotics requires antibiotic control, public health measures and the need to always carry out antibiotic susceptibility test on various isolates before commencing treatment.

1.0.Introduction

Aa Urinary tract infections (UTIs) are the most common bacterial infections, affecting 150 million people each year worldwide [1]. In 2007, in the United States alone, there was an estimated 10.5 million cases for UTI symptoms and about 2–3 million emergency visits [2, 3]. while in Nigeria 150 million of UTIs is reported per annum worldwide [4]. Urinary tract infections are a significant cause of morbidity in in all ages.

Clinically, UTIs are categorized as uncomplicated or complicated [5]. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities; these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) [6]. Several risk factors are associated with cystitis, including female gender,

a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, pregnancy [5, 7]. In the United States, 70–80% of complicated UTIs are attributable to indwelling catheters, accounting for 1 million cases per year [2].

Urinary tract infections are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC), others include *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. [3, 6, 8, 9]. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* [10, 11, 12].
Patients suffering from a symptomatic UTI are commonly treated with antibiotics; these treatments can result in long-term alteration of the normal microbiota of the vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms [13]. The availability of niches that are no longer filled by the altered microbiota can increase the risk of colonization with multidrug-resistant uropathogens. Importantly, the 'golden era' of antibiotics is waning, and the need for rationally designed and alternative treatments is therefore needed. Recent studies have used RNA sequencing to directly analyse uropathogens from the urine of women experiencing symptomatic UTIs. The aim of this study was to isolate UTI organisms and to determine their antibiotic susceptibility pattern among apparently healthy students of University of Benin, Benin city, Nigeria.

2.0. Methodology

2.1. Study Area

This study was conducted in University of Benin, Benin City, Edo State. University of Benin was founded in 1970 as an Institute of Technology and was accorded full University status by the Nigeria National Universities Commission" (NUC) on the 1st of July,1971. The university is located in Benin City, south-south of Nigeria on $6^{0}20.022^{1}$ North latitude and $5^{0}36.009^{1}$ East longitude. It is situated approximately 40km North of the Benin river and 320km by road East of Lagos.

2.2. Sample Collection

Informed consent was obtained from University of Benin Ethical Clearance Committee. Students were counseled about the research work and consented students were given labeled sterile universal bottles to submit their urine sample. The specimen was appropriately labeled, transported to the laboratory, and were analyzed after collection.

2.3. Bacterial Enumeration and Identification

The media used were prepared following manufacturer's specification. Sterilization of glassware and other autoclavable materials was done at 121°C for 15 min. Urine samples were cultured using Cysteine Lactose Electrolyte Deficient (CLED) Agar with Andrade Indicator, Eosin methylene

blue (EMB) and Manitol Salt Agar and incubated for 24 hours at 37 °C. Upon incubation, total bacterial count (TBC) was carried out. The isolates were further identified using cultural and biochemical methods as described by Cheesbrough [14].

2.4. Antibiotics Susceptibility Test and Multiple Antibiotic Resistance (MAR) index

Antibiotic susceptibility of the isolates was determined using the modified Kirby-Bauer disc diffusion technique. A suspension of each isolate was inoculated onto Mueller Hinton agar at 37 °C for 24 hours. The plates were allowed to set and the antibiotic sensitivity disc (ABTEK, India) containing; OFL- Ofloxacin (5µg), AUG- Augmentin (30µg), AM- Amoxicillin (30µg), CXM- Cefixime (5µg), CPR- Ciprofloxacin (5µg), CAZ- Ceftazidime (30µg), CRX- Cefuroxime (10µg), GEN- Gentamicin (30µg). The plates were incubated at 37 °C for 24 hours and the resultant zone of inhibition were measured and recorded. The obtained results were interpreted based on the guidelines of the Clinical Laboratory Standard Institute [15].

MAR index is a handy tool used in assessing and identifying health risk factors caused by isolates either from high or low use of antibiotics. The MAR index, when applied to a single isolate, is defined as $^{a}/_{b}$, where a represents the number of antibiotics to which the isolate was resistant, and b represents the number of antibiotics to which the isolate was exposed as described by Krumperman [16].

3.0. Results

Table 1 shows the total percentage distribution of urinary tract bacteria isolated from urine samples of apparently health student of University of Benin. One hundred and fifty (150) samples of urine were obtained from male and female students of UNIBEN and Eosin methylene blue (EMB) agar had the highest occurrence of microorganism present in the urine, followed by Cysteine Lactose Electrolyte Deficient (CLED) agar and mannitol salt agar. In CLED agar the predominant isolate was *E. coli* consisting of105 (34.54%), followed by *Staphylococcus aureus* 95 (31.25%), *Klebsiella pneumonia* 67 (22.04%) and *Proteus mirabilis* 37 (12.17%).

The percentage frequency of occurrence of bacteria isolated from urinary tract of apparently healthy students based on gender is presented on Table 2. The result revealed that *Escherichia coli* had a percentage of (42.54%), *Staphylococcus aureus* (34.25%), *Proteus* spp (12.71%), *Klebsiella* spp (10.50%) in female, while in male the percentage occurrence was; *Klebsiella* spp (39.02%), *Staphylococcus aureus* (26.83%), *Escherichia coli* (22.76%) and *Proteus* spp (11.38%).

Ten (10) isolates each of *E. coli*, *K. pnuemoniae*, *P. mirabilis* and *S. aureus* were tested for antibiotic susceptibility using commercially purchased antibiotic discs. All the ten (10) isolates of *E. coli* were resistant to AM- Amoxicillin (30µg), while eight isolates were susceptible to GEN- Gentamicin (30µg). *P. mirabilis* recorded the highest susceptibility to OFL- Ofloxacin (5µg), CPR- Ciprofloxacin (5µg), CAZ- Ceftazidime (30µg), CRX- Cefuroxime (10µg), GEN- Gentamicin (30µg). Also all the isolates of *K. pnuemoniae* were seen to be susceptible to AUG- Augmentin (30µg) as shown on Table 3. Table 4 shows the Multiple Antibiotics Resistant (MAR) index of all the isolates from the study population. All the isolates were resistant to more than two antibiotics with MAR index of greater than 0.2 (>0.2).

Table 1: Total percentage distribution of Bacteria isolated from Urine Samples of Apparently Health Student of University of Benin

Bacteria Isolates	CLED Agar		EMB Agar		Mannitol Agar	
	Occurrence (n)	Frequency (%)	Occurrence (n)	Frequency (%)	Occurrence (n)	Frequency (n)
E. coli	105	34.54	379	100	0	0
S. aureus	95	31.25	0	0	267	100
P. mirabilis	37	12.17	0	0	0	0
K. pnuemoniae	67	22.04	0	0	0	0
Total	304	100.00 %	379	100.00 %	267	100.00 %

Table 2: Percentage frequency of occurrence of Bacteria isolated from urinary tract of apparently healthy students based on gender.

Bacteria Isolates	Male		Female	
	Occurrence (n)	Frequency (%)	Occurrence (n)	Frequency (%)
E. coli	28	22.76	77	42.54
S. aureus	33	26.83	62	34.25
P. mirabilis	14	11.38	23	12.71
Klebsiella spp	48	39.02	19	10.50
Total	123	100.00 %	181	100.00 %

3.1. Discussion

Urinary tract infections (UTIs) are one of the most common bacterial infections in the human urinary system. *Escherichia coli* was recorded as one of the prevalent isolate in this study, it agrees with the report on uropathogens showing *E. coli* as the most frequently isolated organism in patients with UTIs [17, 18]. The high incidence of *E. coli* is attributed to the fact that it is a commensal of the bowel and infection is mostly through feacal contamination occasioned by poor hygiene. Also, improper wiping after urination or defecation can result in transfer of organisms from the anus to the distal urethra.

The most predominant bacterial isolate from male is *Klebsiella* spp (39.02 %) followed by *Staphylococcus aureus* (26.83%) and the least occurrence was *Proteus mirabilis* (11.38 %) while female is *Escherichia coli* (42.54 %) followed by *Staphylococcus aureus* (34.25 %) and the least

was *Proteus mirabilis* (12.71 %). This work is in agreement with the work of Al-Hilali (2018) and Anuli *et al.* [19, 20] who reported *E, coli* as the predominant bacteria isolated among students. This finding is also similar to the finding of Akortha and Ibadin [21] which shows Gram-positive bacteria, particularly *S. aureus* as one of the most commonly implicated pathogen in patients with UTIs. This is as a result of its minimal growth requirements, ability to survive long in most unfavourable environments and to find a susceptible host. Akortha and Ibadin [21], noted that the high incidence of Gram positive bacteria are possibly due to the virulent nature of the organism, which gave it the ability to overcome body defense mechanisms and resistance to antibiotics. It was suggested that *S. aureus* is the most frequently isolated organism as well as the leading etiologic agent in urinary tract infection in our environment [22]. It constituted as high as 16 % of cases in women suspected of UTI compared to men (10.7%) and in agreement with the findings presented by Abdul and Onile [23] showing UTI caused by *S. aureus* as the most common among women in Ilorin, North-central, Nigeria. The high incidence of *S. aureus* in women could be due to the proximity between the genital tracts and the urethra/anus, which perhaps facilitate auto transmission as earlier reported [24].

Table 3: Antibiotic Susceptibility Profile of Bacteria Isolates from Urinary Tract of apparently healthy students

Probable Isolate	Sensitivity	Antibiotics (mm)							
		OFL	AUG	AM	CPR	CAZ	CRX	GEN	CXM
E. coli	S	7	1	-	3	5	-	8	2
	I	3	3	-	1	1	6	-	2
	R	-	6	10	6	4	4	2	7
K. pnuemoniae	S	8	10	-	3	1	1	2	2
	I	2	-	-	5	2	3	2	3
	R	-	-	10	2	7	6	6	5
P. mirabilis	S	10	10	-	10	10	10	10	2
	I	-	-	-	-	-	-	-	2
	R	-	-	10	-	-	-	-	6
S. aureus	S	5	8	7	1	8	4	6	1
	I	3	2	2	3	2	3	4	3
	R	-	-	1	6	-	3	-	6

KEY: S= susceptible, I= intermediate, R= resistant, OFL- Ofloxacin 5µg, AUG- Augmentin 30µg, AM- Amoxicillin 30µg, CXM-Cefixime5µg, CPR- Ciprofloxacin 5µg, CAZ- Ceftazidime 30µg, CRX- Cefuroxime 10µg, GEN-Gentamicin30µg

Table 4: Antibiotic Susceptibility Profile and MAR index of Methicillin Resistant *Staphylococcus aureus* isolated from urine samples

Isolates	Resistance profile	Number of resistance	MAR index
E. coli	AUG, AM, CPR, CAZ, CRX, GEN, CXM	7	0.9
K. pnuemoniae	AM, CPR, CAZ, CRX, GEN, CXM	6	0.8
P. mirabilis	AUG, AM, CXM	3	0.4
S. aureus	AM, CPR, CRX, CXM	4	0.5

KEY: OFL- Ofloxacin 5µg, AUG- Augmentin 30µg, AM- Amoxicillin 30µg, CXM-Cefixime5µg, CPR- Ciprofloxacin 5µg, CAZ- Ceftazidime 30µg, CRX- Cefuroxime 10µg, GEN-Gentamicin 30µg.

Antibiotic susceptibility profile was recorded with all eight (8) isolates observed to have MAR Index of greater than 0.2. The high incidence of *E. coli* is attributed to the fact that it is a commensal of the bowel and infection is mostly through faecal contamination occasioned by poor hygiene which was observed to be resistant to Augmentin, Amoxicillin, Cefixime, Ciprofloxacin, Ceftazidime, Cefuroxime, Gentamicin. This is similar to the work of Olson and Haith [25], who reported *E. coli.* to be resistant to Ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, amoxicillin/clavulanate. Also all the isolates were observed to be resistant to amoxicillin but susceptible to ofloxacin, this is in line with the work of Ayoade et al. [26] among university students Ogun State, Nigeria. Mordy and Erah [27], also reported resistant of microorganisms to amoxicillin. This could be attributed to the antibiotic being relatively cheaper, easily available leading to it high purchase and its high prescription rate.

It was found that all the isolates were resistant to more than two antibiotics and recorded MAR index ≥ 0.2. This could be attributed to the isolates probably from high-risk contamination source, heavily use of antibiotics. World Health Organization had invited all countries to adopt strategies to control, monitor and prevent antimicrobial resistance [28]. Nigeria needs to set up a national agency on antimicrobial resistance to collect, track and report antimicrobial resistance trends to monitor the free access to antibiotics in Nigeria. To contain antibiotic resistance, culture and susceptibility test should be undertaken, before prescriptions are given, with strict national quality assurance program for reporting laboratories and educating health professionals and populace on the public health effect of antibiotics resistance.

4.0. Conclusion

In this study, urinary tract infection appears to be more among the female students in the University of Benin, Ugbowo than the male students and the increase in resistance of the isolates to the antibiotics should require the need to always carry out antibiotic susceptibility test of various isolates before commencing treatment.

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