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Distribution and Antimicrobial Susceptibility Profile of Bacterial Isolates in Males and Non- pregnant Females with Urinary Tract Infection in University of Benin Health Centre, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FIA designed the study, carried out laboratory analysis in conjunction with author VEO, financed the research and wrote the manuscript. Author VEO carried out sample collection and laboratory analysis under the supervision of author FIA, sourced for relevant journals and participated in writing-up. Both authors read and approved the final manuscript.

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ABSTRACT

Urinary tract infections (UTIs) have become the most common hospital and community acquired infections resulting in high morbidity. The prevalence of bacteria causing UTI as well as their susceptibility to commonly used antibiotics was investigated at the University of Benin Health Centre (UBHC), Benin city, Nigeria. Bacterial species isolated from the urine samples analyzed were *Escherichia coli*, *Klebsiella* sp. *Enterococcus* sp., *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. UTIs in this study, was found to be more prevalence in females (60% for 15- 26 years and 53% for 27- 32 years) than in males (40% for 15- 26 years and 47% for 27-32 years). The isolates were identified by carrying out standard biochemical tests and comparing with characteristics of known taxa. Susceptibility tests were performed by Bauer-Kirby Disc diffusion method with eleven antibiotics. The results were expressed as susceptible / resistant. Gram-negative isolates had a prevalence of 66.7%, while Gram-positive isolates had 33.3%. *E. coli*

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were found to be the most prevalence occurring in 80% of the samples, while the least prevalent were *Pseudomonas aeruginosa*. *Staphylococcus* sp and *Enterococcus* sp occurred in 40% of the samples. All isolates were found to be susceptible to ciprofloxacin. Gram-positive isolates were 100% resistant to Penicillin, while the Gram-negative isolates were 100% resistant to Amikacin.

Keywords: Urinary tract infections; multi-drug resistance; susceptibility profile; prevalence; disc diffusion.

1. INTRODUCTION

Urinary tract infections are serious health problems affecting millions of people each year. They are the second most common type of infections in the body. UTIs are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings and ranks high amongst the most common reason that compel an individual to seek medical attention [1,2,3,4]. It encompasses a spectrum of clinical entities ranging in severity from asymptomatic infections to acute cystitis, Prostatitis, Pyelonephritis and Urithritis [5,6]. It represents one of the most common diseases encountered in medical practice today, affecting people of all ages from the neonatal to the geriatric age group [7,4].

UTIs occur more often in women than men because a woman's urethra is shorter and wider and is more readily transverse by microorganisms [8] the short urethra makes it easier for bacterial from the anus or genital area to reach the bladder. The structure of the female urethra and vaginal makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra and into the bladder during pregnancy and or child birth [3,4,9]. In pregnancy the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine [10]. Worldwide about 150 million people are diagnosed with UTIs each year [11].

Majority of UTIs are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia [12]. Transmission occurs in four ways; namely through sexual intercourse, from mother to the foetus via placenta, through poor personal hygiene and via communal sponge and towel usage [13].

UTI is described as a bacteriuria with urinary symptoms. It can affect lower and sometimes both lower and upper urinary tracts. The term cystitis has been used to define the lower UTI infection and is characterized by symptoms such as dysuria frequent urination and suprapubic tenderness. The presence of the lower UTI symptoms does not exclude the upper UTI which is often present in most UTI cases [14]. The spectrum of bacteria causing complicated UTI is much broader than those causing uncomplicated UTI. However, the most commonly encountered microorganisms are Gram-negative bacteria including *Escherichia coli, Citrobacter sp, Enterobacter aerogenes, Pseudomonas aeruginosa* and *Proteus vulgaris* whereas *Klebsiella* sp, *Staphylococcus aureus* and *Salmonella* sp. are found rarely [15].

Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem. In the last three decades there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections [16,17,18]. Also the rising prevalence of drug resistance such as penicillin-resistant Pneumococci worldwide mandate selective susceptibility testing and epidemiological investigations during outbreaks [19]. Epidemiological surveillance of antimicrobial resistance is indispensible for empirically treating infections implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms [20,19]. The changing pattern in the etiological agents of urinary tract pathogens and their sensitivities to commonly prescribed antibiotics are reported [21,17,18,22].

The worldwide escalation in both community and hospital acquired antimicrobial resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and new treatment alternatives [23,24,25,19]. UTI is serious issues particularly in the developing world were apart from high level of poverty ignorance and poor hygiene practices there is

also high prevalence of fake and spurious drugs of questionable quality in circulation.

This study therefore was undertaken to determine the prevalence of UTI in male and female patients, the distribution of microorganisms among the different ages and genders and their antimicrobial susceptibility profile in UBHC.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh mid-stream urine was collected from each patient in University of Benin Health centre, Benin city into a 20 ml calibrated sterile screw-capped universal container which was distributed to the patients. The specimens were labelled transported to the laboratory and analyzed within 6 hours. In each container boric acid (0.2 mg) was added to prevent the growth of bacteria in urine samples. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Verbal informed consent was obtained from all patients prior to specimen collection. The study was conducted after due ethical approval.

2.2 Isolation and Identification of Isolates

The samples were inoculated on MacConkey agar and Blood agar. The inoculum on the plate was streaked out for discrete colonies with a wire loop following standard procedures [26,22]. The culture plates were incubated at 35-37ºC for 24 hours and observed for growth through formation of colonies. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel [27] and Cheesbrough [26].

2.3 Antibiotic Susceptibility Test

This was carried out using the Kirby-Bauer disc diffusion method [28], according to NCCLS [29], and using antibiotics containing discs from Oxoid. 20 ml of the Muller-Hinton agar was prepared and poured into sterile plates. The agar medium was allowed to solidify at room temperature on a flat bench. Isolates were streaked on the surface of the well dried agar plates. The antibiotics disc were gently and firmly placed on the agar plates which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 35-37ºC for 24 hours. Zones of growth inhibition were then measured to the nearest millimeter and recorded. The mean of triplicate results was taken as the zone of diameter.

Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested [20]. Gram positive and Gram negative multi disc were used. The antibiotics used for the isolates were: Amikacin (30 µg), Cefalexin (10 µg), Cefuroxime (10 µg), Amoxicillin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Vancomycin (10 µg), Nitrofurantoin (20 µg), Tetracycline (10 µg), Penicillin (20 µg), cefpodoxime (20 µg).

3. RESULTS AND DISCUSSION

This study was carried out to evaluate the prevalence and susceptibility patterns of bacterial strains, isolated from patients diagnosed with UTI in University of Benin Health centre Benin city. Microorganisms and their resistance patterns vary from hospital to hospital and even from clinic to clinic in the same hospital [30,31] We also investigated the relationship between gender (Males and Females) and the isolated bacterial UTI agents. This study was confined to young males and non-pregnant females with UTI in UBHC.

The results of this study showed that the etiologic agents of UTIs mainly belonged to the gram negative bacteria, with Escherichia coli being the most prevalence as seen in Fig. 1. This agrees well with the previous studies conducted [32,33, 34,35]. The disease was found to be more prevalent in females (60% for 15-26 years and 53% for 27-32 years) than in males (40% for 15-26 years and 47% for 27-32 years) in this study Fig. 2. The sex distribution of patients in this study was consistent with that of other studies [36,37,38,39,30,40]. Several reports have indicated that females are more prone to having UTIs than males [4], because the urethra is shorter in females than in males and it's easily transversed by microorganisms [8].

The tendency of women to develop UTI, has been explained to depend on factors such as pregnancy and sexual activity [41,42,43,4] bad toilet as well as the use of diaphragm [44,45,19]. Also the length of the urethra the dried environment surrounding the meatus, and the antibacterial properties of prostatic fluid contribute to a lower rate of infection in males. Women before the age of 24 years are more prone and suffer from recurrent UTI than men [46,47,48].

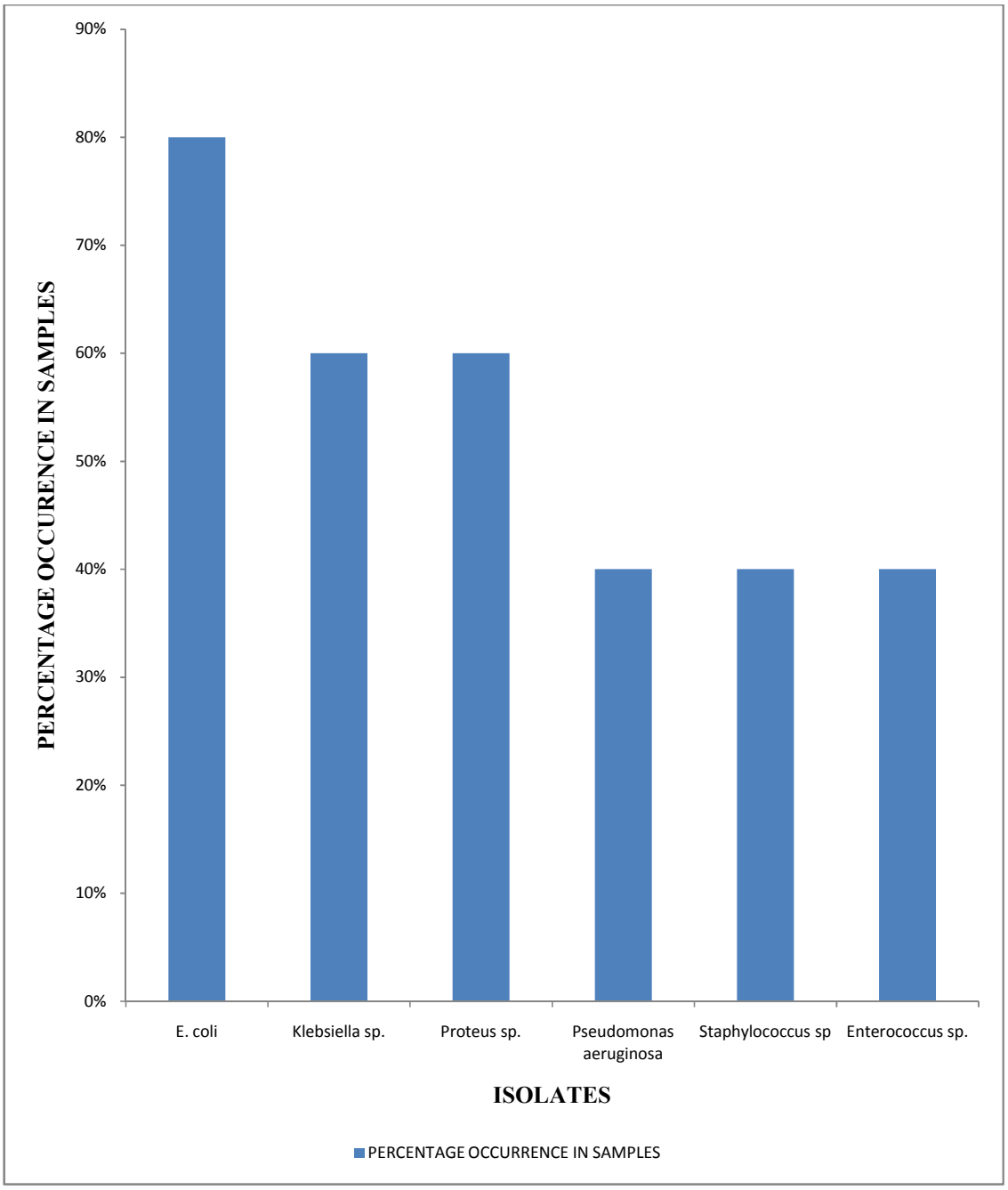

Fig. 1. Distribution of bacterial isolates among samples

The uropathogens isolated and identified in this study (Table 1), were similar to those of many other studies conducted in different countries, either in the region or internationally [32,46]. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions, and host factors practices such as health care and education programmes, socioeconomics standards and hygiene practices in each country. The Enterobacteriaceae family were the most common microorganisms isolated from UTI. In this study (Table 1), amongst them *E. coli* was the most predominant occurring in 80% of the samples, closely followed by *K. pneumoniae* occurring in 60% of the samples. This is in

agreement with the result of Mordi and Erah, [22]; Dash et al. [49]; Abubakar, [40]; Johnson et al. [34]; Omigie et al. [50]. This high incidence of *E. coli* and other Enterobacteriaceae could be attributed to the fact that they are commensals of the bowels and that infections is mostly by faecal contamination due to poor hygiene. This is owned to the fact that commensals of the intestines are more involved in the UTI because of the anatomy proximity to the genitourinary area [46]. This is contrary to reports in which *P. aeruginosa* and *Klebsiella* sp. [51] which was recorded as the predominant bacteria in UTI. Another reason for the high incidence of Gram-negative bacteria related to Enterobacteriaceae in causing UTI is that they have many factors responsible for their attachment to the uroepithelial and they are able to colonize in the urogenital mucosa with adhesions, pilli, fimbriae and P-1 blood group phenotype receptor [52].

Table 1. Isolated bacteria from samples

Bacterial isolates
Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis
Pseudomonas aeruginosa
Staphylococcus aureus
Enterococcus faecalis

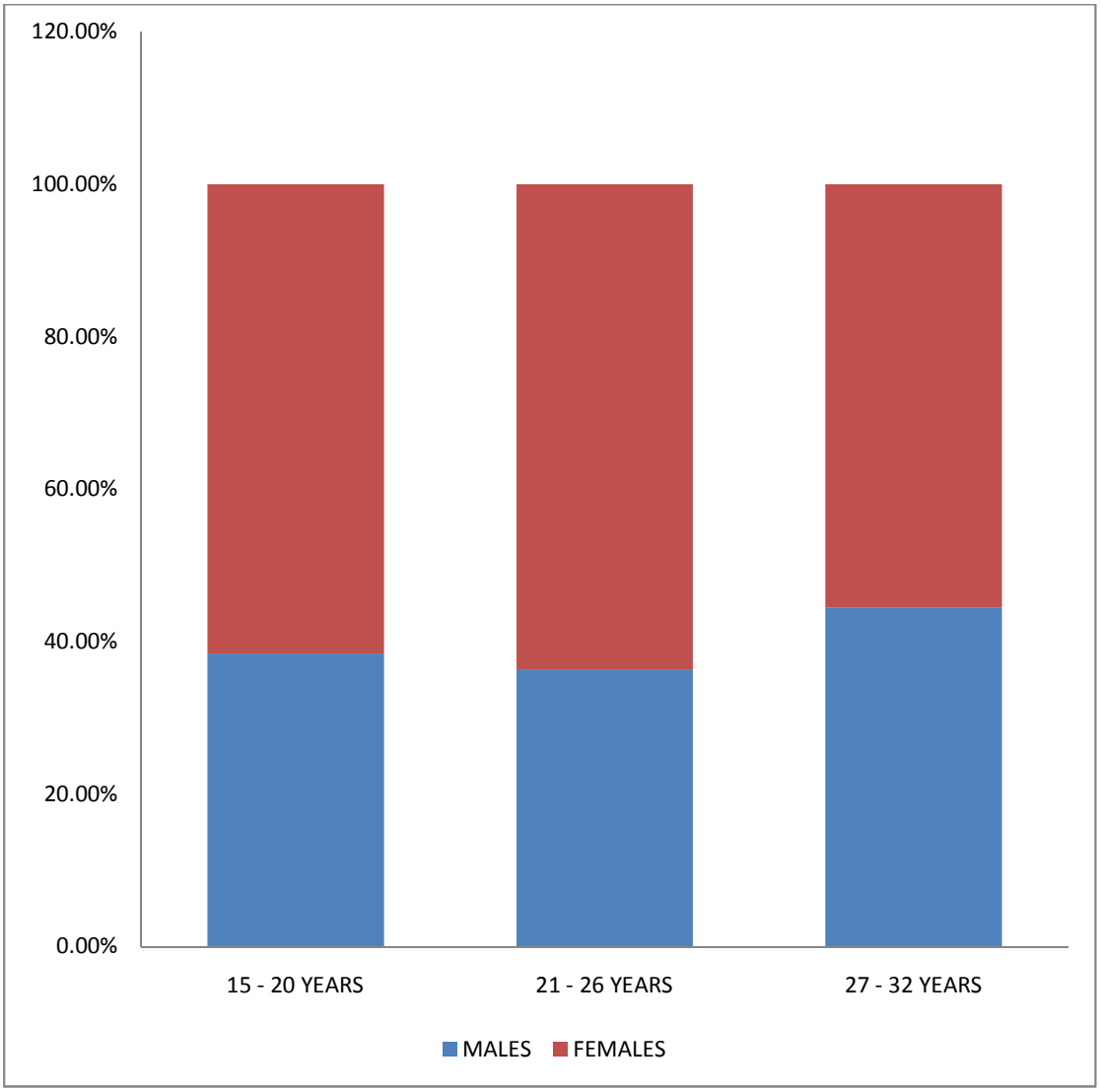

Fig. 2. Age and sex distribution of UTI patients

Table 2. Antibiotic sensitivity pattern of gram-negative isolates in UBHC

Organisms	Antibiotics					
	Amk	**Cef**	**Cfx**	**Cip**	**Nft**	**Cpd**
E. coli	S	R	R	S	S	R
Klebsiella sp	S	R	S	S	S	S
P. aeruginosa	S	R	S	S	R	R
P. mirabilis	S	R	R	S	R	S

Where: Amk= Amikacin; Cip = Ciprofloxacin; Cef = Cefalexin; Nft= Nitrofurantoin; Cfx= Cefuroxime; Cpd = Cefopodoxime; S= Sensitivity; R= Resistant

Table 3. Antibiotic sensitivity pattern of gram-positive isolates in UBHC

Antibiotics	Gram-positive Isolates	
	Staphylococcoccus aureus	***Enterococcus faecalis***
Amoxicillin	S	S
Chloramphenicol	R	S
Ciprofloxacin	S	S
Vancomycin	S	S
Nitrofurantoin	S	S
Tetracycline	S	S
Penicillin G	R	R

Where: R= Resistant; S= Sensitivity

The results of antibiotics susceptibility test on gram negative bacteria showed that *E. coli* was resistant to cefalexin, cefuroxime and cefpodoxime (Table 2) and they show a high degree of susceptibility to Amikacin, nitrofurantoin and ciprofloxacin. This goes in agreement with earlier studies by Wazait *et al*. [53]. Gram negative isolates showed 100% susceptibility to ciprofloxacin. This result is similar to that reported by Resih et al. [54] and Okonko et al. [19]. Klebsiella sp. was found to have high resistance to cefalexin and susceptibility to nitrofurantoin and ciprofloxacin. This is in agreement with the report of Wazait et al. [53]. Pseudomonas aeruginosa was found to be resistant to cefalexin, cefpodoxime and nitrofurantoin. This is similar to the results of Mazzulli et al. [55]. *P. aeruginosa* maintains antibiotic resistance plasmids and are able to transfer these genes by bacterial processes by transduction and conjugation [56]. *Proteus mirabilis* showed resistance to cefalexin, cefuroxime and nitrofurantoin while being susceptible to Amikacin, ciprofloxacin and cefpodoximen (Table 2). *Proteus* sp has the ability to produce urease which helps them to survive in the urinary tract, and they have flagella that enable them to swim about their environment and also contribute to their virulence nature [57,34,32,58]. In this study therefore the most effective antimicrobial agent against all the gram negative and gram positive bacteria isolated from UTI patients in UBHC was found to be ciprofloxacin.

Staphylococcus aureus and *Enterococcus faecalis* showed a high resistance to penicillin G and high susceptibility to nitrofurantoin, amoxicillin, ciprofloxacin, vancomycin and tetracycline with *E. faecalis* showing susceptibility to chloramphenicol as compared to *Staphylococcus aureus* (Table 3). These findings were similar to those observed by Nkang et al. [59] but contrary to Bischoff et al. [60]. This might be due to environmental differences. *S. aureus* has been reported to exhibit resistance to beta-lactam antibiotics of which benzyl penicillin is one Outbreaks of *S. aureus* resistant to beta-lactam antibiotics have been frequently associated with devastating nosocomial infections [61,62,63,25].

Multidrug resistance was observed for some of the isolates as they were resistant to more than one drug [64,65]. Drug abuse and indiscriminate misuse of antibiotics among the general population has favoured the emergence of resistant strains.

4. CONCLUSION

The susceptibility and resistance profile of all isolates in this study have shown that ciprofloxacin is the most effective drug of choice. The findings of this study confirm that still some

bacteria are resistant to antibiotics and frequently used drugs in many parts of the world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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