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THE ANTIBIOGRAM STUDIES OF *STAPHYLOCOCCUS* ISOLATES FROM URINE SAMPLES OF PATIENTS WITH URINARY TRACT INFECTIONS IN ESAN, EDO-NIGERIA

***[1]Momoh ARM., [2]Adewuyi G., [3]Morgan E., [4]Orhue PO., [1]Okolo PO.,**

[1]Department of Medical Microbiology, Ambrose Alli University, Ekpoma.

[2]Department of Medical Microbiology, Irrua Specialist Teaching Hospital, Irrua.

[3]Department of Surgery, Irrua Specialist Teaching Hospital, Irrua.

[4]Department of Medical Laboratory Sciences, Ambrose Alli University, Ekpoma.

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***Corresponding Author**
Momoh ARM.
Department of Medical Microbiology, Ambrose Alli University, Ekpoma.

ABSTRACT

This study aimed to determine the antibiogram of *Staphylococcus* species isolated from confirmed urinary tract infected individuals in Esan-Nigeria. A retrospective analysis was done in the period of January 2013 to December 2013 in three health facilities in the study area. Twenty-one antibiotics susceptibility testing of *Staphylococcus* species pathogens isolated from urine samples of male and female patients were assessed. 186 urine samples made up of 101 male (consisting of 92 *S. aureus* and 9 *S. saprophyticus*) and 85 female (consisting of 72 *S. aureus*, 7 *S. saprophyticus* and 6 *S. epidermidis*) samples were analyzed. Overall, S. aureus isolates from male urine sample were most sensitive to augmentin and azithromycin while the female samples were most sensitive to noreloxcine and gentamycin. Male urine S. saprophyticus isolates were most sensitive to ofloxacin and resistant to 10 different antibiotics while that of the female were most sensitive to augmentin, noreloxcine and ciprofloxacine and resistance to 12 antibiotics. Female urine S. epidermidis isolate was most sensitive to noreloxcine and resistance to 12 antibiotics. Based on the findings, in the study area, S. *saprophyticus* and *S. epidermidis* are multi-drug resistant and antibiotic treatment of UTI in this area faces serious challenge.

KEYWORDS: Urinary tract infection, *Staphylococcus* species, Antibiogram, Esan, Nigeria.

INTRODUCTION

Microbiologically, urinary tract infection exists when pathogenic microorganisms are detected in the urinary tract.[1, 2] Urinary tract infection (UTI) applies to a variety of clinical conditions ranging from asymptomatic urine presence of bacteria to severe kidney sepsis.[3] This is despite the presence of several antibacterial factors in urine such as pH, urea concentration, osmolarity, various organic acids, salt content of the urine, bacterial adherence inhibitors (such as Tamm-Horsfall protein or THP, bladder mucopolysaccharide, low molecular weight oligosaccharide, secretory IgA and lactoferrin), the uropathogenic bacteria are able to adhere, grow and resist against host defenses which finally result in colonization and infection of the urinary tract.[4-6] Both in the community and hospital settings, UTI is one of the most common bacterial infections in humans[7] and the most common bacterial infections encountered by clinicians in developing countries.[8] Approximately 150 million people are diagnosed and over USD 6 billion health care expenditures goes to UTI Worldwide.[3]

The etiological agents and susceptibility patterns of UTI vary in regions and geographical location[9] and change through time.[10] Drug resistance among bacteria causing UTI has increased since introduction to UTI chemotherapy.[11-15] By implication, the challenge in treatment of UTI with antibiotics and more serious challenge in developing countries where self medication is ramped. In this regards, Leegaard et al.[16] suggested the need for knowledge of the local bacterial etiology, and their susceptibility patterns to trace any change. This suggestion is important as it can identify any change that might have occurred over time and also provide updated recommendation for optimal UTI treatment and as such minimized drug resistant.

*Escherichia coli***;** which usually can be found in gastrointestinal tract, has been the most common agents for UTI[17], other bacteria like *Staphylococcus* spp. causes such infections today and the prevalence is increasing.[18] In fact, species, like *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* cause UTI in young and old, men and women but rarely in children.[17-20] The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health problem particularly in the developing World where apart from high level of poverty, ignorance and poor hygiene practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation.[21] Hence, the changing spectrum of microorganisms involved in urinary tract

infections and emergence of resistance across institutions and geographical areas have made imperative the conduct of antibiotic susceptibility testing study of UTI pathogens in various regions from time to time. Besides, the need for constant monitoring of susceptibility of specific pathogens in different populations to commonly used antimicrobial agents has been suggested.[22] The aim of this present study was therefore to study the antibiogram profile of *staphylococcus* species isolates from urine samples of patients with urinary tract infections in Esan, Edo-Nigeria.

MATERIALS AND METHODS

2.1 Specimen: A total of 186 specimens comprising 101 male and 85 female Mid-Stream urine specimen of in and out patients in Irrua Specialist Teaching Hospital (ISTH), Irrua in Esan Central Local Government Area, and Gilead Hospital, Ekpoma and Calvary Medical Centre, Ekpoma, both in Esan West Local Government Area, in Edo State, Nigeria. Samples were obtained from 5th January 2013 to 20th December 2013.

2.2 Isolation and identification: Standard microbiological analysis was carried out on these samples. The specimens were inoculated onto nutrient agar, blood agar and MacConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37oC for 24 hours. After 24 of incubation, discrete colonies were picked up and gram stained and further sub-culturing was done to obtain pure cultures and biochemical tests carried out.

2.3. Antibiotics under Study: twenty-one antibiotics as previously reported in Momoh et al.,.[23]

2.4 Antibiotics susceptibility testing (Antibiogram): This was done by the multi-discs diffusion using 21 different antibiotics. The multi discs were placed on the plates which were previously inoculated, few minutes earlier, then the plates were incubated at 37oC for 24 hours, thereafter, the plates were examined for zones of inhibition around the different antibiotic disc. *Staphylococcus aureus* Oxford stain NTC 6751 was used as control for Gram positive organisms.

2.5 Mnemonic Coding: The Ajumali's mnemonic coding method as earlier described by Joghi et al.[24], was adopted as a typing scheme to re-arrange the nominal antibiotics into arbitrary numeric values, making it easy for the differentiation of strains. Using this pneumonic coding scheme, a sensitive result was scored as (+), while a resistance was scored

as (-). Also, the 21 different antibiotics were divided into a group of 3 antibiotics each, following their mechanisms of action as well as; their clinical applications; and these 3 antibiotics were given numerical values of 1, 2 and 4.

Thus, a perfect sensitivity to the 3 antibiotics will give a summation of 1+2+4 =7. While complete resistance to the 3 antibiotics will give a summation of 0+0+0 = 0. The other values as obtained by adding up these numerical values thus, an isolate can receive a score of 0-7 in each triplet segment, which, when the seven triplet segments are combined together, gives a seven (7) digit numerical value as the antibiogram types.[25]

2.6. Data analysis: All data were analyzed using simple descriptive statistic.

RESULTS

Table 1 shows the frequencies of the isolated *staphylococcus* species from urine samples of male and females with UTI. *Staphylococcus aureus* (n= 92 vs. 72) and *Staphylococcus saprophyticus* (n= 9 vs. 7) were more prevalent in male urine samples while only the female urine samples presented positive isolates of *Staphylococcus epidermidis* (n=6).

Table 1: Frequency of isolated *staphylococcus* species from urine samples of male and females with UTI.

ORGANISMS	Number of isolates		Total
	Male	Female	
Staphylococcus aureus	92 (56.10)	72 (43.90%)	164
Staphylococcus saprophyticus	9 (56.25%)	7 (43.75%)	16
Staphylococcus epidermidis	0 (0.0%)	6 (100.0%)	6
Total	101 (54.30%)	85 (45.70%)	186

Figure 1 highlights the antibiogram types of the ninety-two isolated strains of *Staphylococcus aureus* from the male urine sample. Augmentin was the most sensitive while Streptomycin was the least sensitive against *Staphylococcus aureus* isolated from male urine samples. None of the anitibiotics used showed sensitivity higher than 30% on *staphylococcus aureus* isolates from male urine samples. The antibiogram profile (Ajumali's mnemonic coding) indicated that strain 33 (mnemonic code: 0400000) was the most resistant strain as it was sensitive to only one antibiotics (Flucloxacillin). Strains 4 (mnemonic code; 0010001) and strain 34 (mnemonic code: 0010010) were also highly resistant being susceptible to two antibiotics

each. Strain 5 (mnemonic code; 62200110) is among the least resistant strains, being sensitive to 5 different antibiotics.

Figure 2 highlights the antibiogram types of the nine isolated strains of *Staphylococcus saprophyticus* from the male urine sample. Several antibioties were 100% resistance while only Ofloxacin was over 50% sensitive (5/6) (see figure 2), with strain 3 (mnemonic code: 0000240) and strain 4 (Mnemonic code: 0010010), showing strains were sensitive to only two groups of antimicrobials, that is, Macrolides and Cephalosporins as well as Gentamicin and Cephalosphorins respectively.

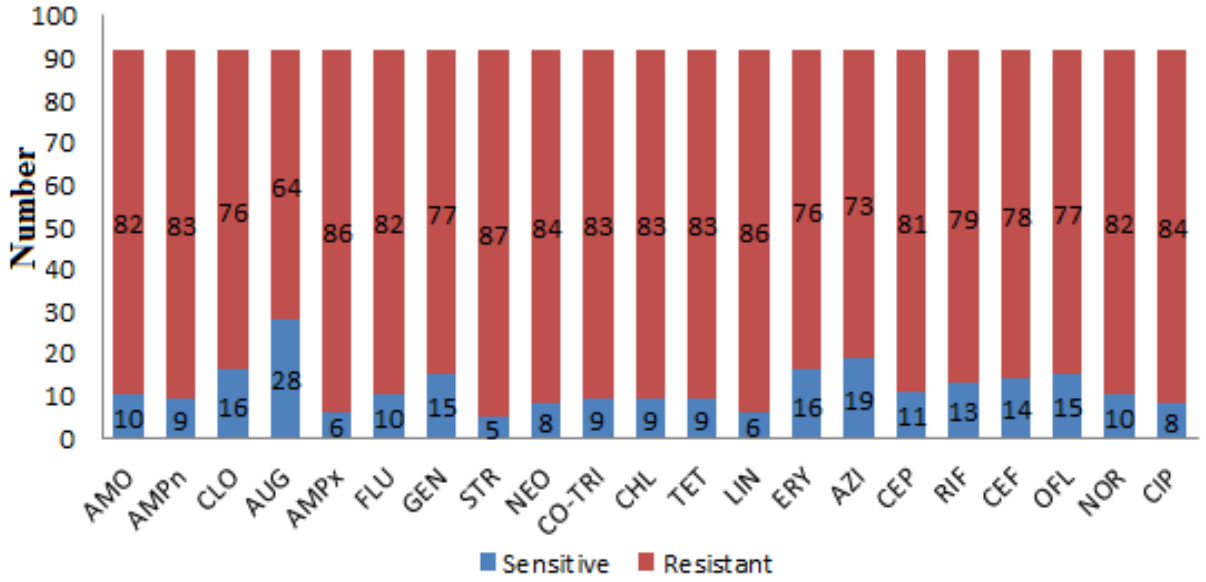

Figure 1: The antibiogram types of *staphylococcus aureus* isolated from male urine samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN= Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxcine, CIP=Ciprofloxacin

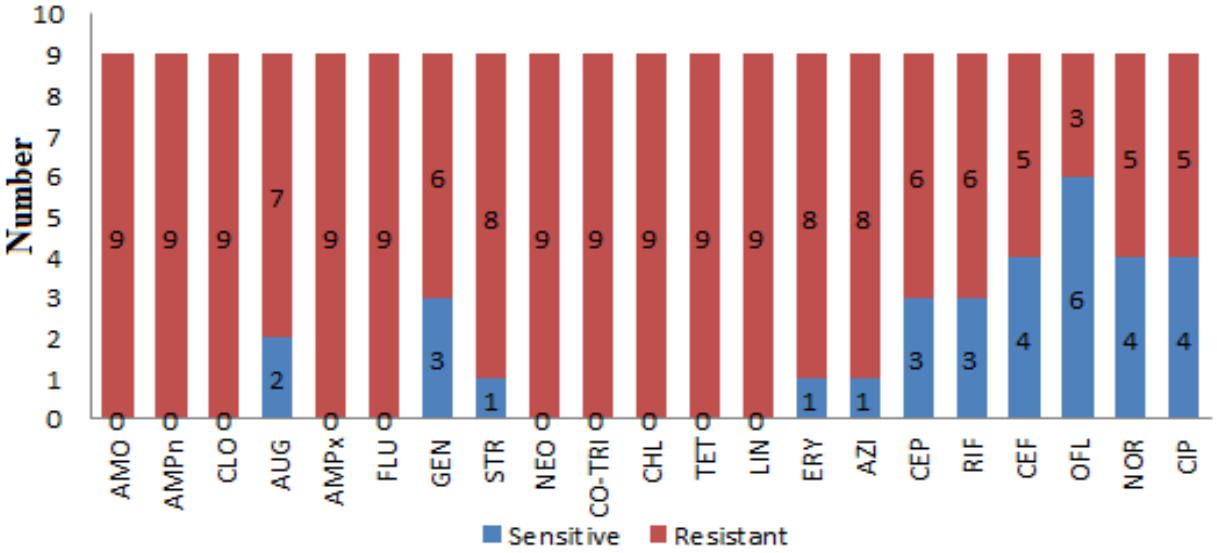

Figure 2: The antibiogram types of *staphylococcus saprophyticus* isolated from male urine samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN=Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxcine, CIP=Ciprofloxacin

Figure 3 shows the antibacterial profiles of all 72 isolated strains of *Staphylococcus aureus* obtained from female urine samples. Norfloxacin was the most sensitive antibiotic (26/72) while Amoxicilin was the least sensitive (1/72). There were similar antibiogram patterns with strains 29, 8, 41, 45, 47, 48, 51, 52 and 57 (Mnemonic codes: 0000020, 0002000, 0000200, 0200000, 0000004, 0000001, 0000002, 0010000, 0000100), showing resistance to all but one antibiotics.

Figure 4 shows the antibacterial profiles of all 7 isolated strains of *Staphylococcus saorophyticus* obtained from female urine samples. *Staphylococcus saprophyticus* from female urine samples showed 100% resistance to several antibiotics. In fact no antibiotic was 50% sensitive. The highest sensitivity (3/7) was shown by Augmentin, Norfloxcin and Ciprofloxacin. The antibiogram typing shows that strains 6, 2 and 3 were the most susceptible, with strain 6 (mnemonic code: 0000207) being susceptible to four antibiotics and strains 2 and 3 were susceptible to three antibiotics, each of these antibiotics were of different pharmacokinetic group.

Figure 5 shows the antibacterial profiles of all 6 isolated strains of *Staphylococcus epidermidis* obtained from female urine samples. It was observed that several antibioties were 100% resistance; while Norfloxacin was the most sensitive with 5 strains sensitive out of the 6 samples and this was followed by Ofloxacin which has 4/6 sensitivity and Gentamycin and Cephalexin presenting 50%. The antibiogram typing reveals that strain 6 (Mnenomic code: 0010010) is the most resistant and was susceptible to two antibiotics. Strain 4 (Mnemonic code: 0030007) was shown to be the most susceptible (to five antibiotics).

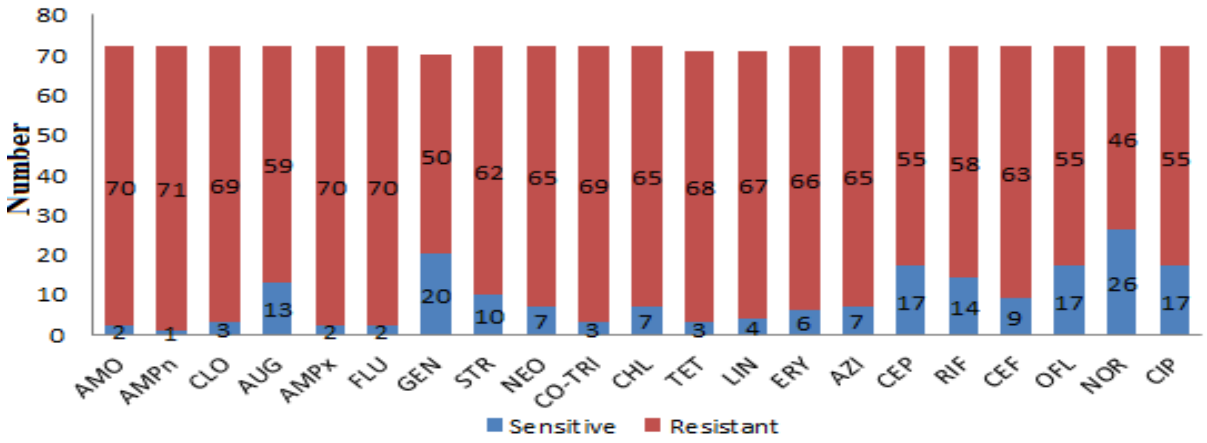

Figure 3: The antibiogram types of *staphylococcus aureus* isolated from female urine samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN=Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxcine, CIP=Ciprofloxacin

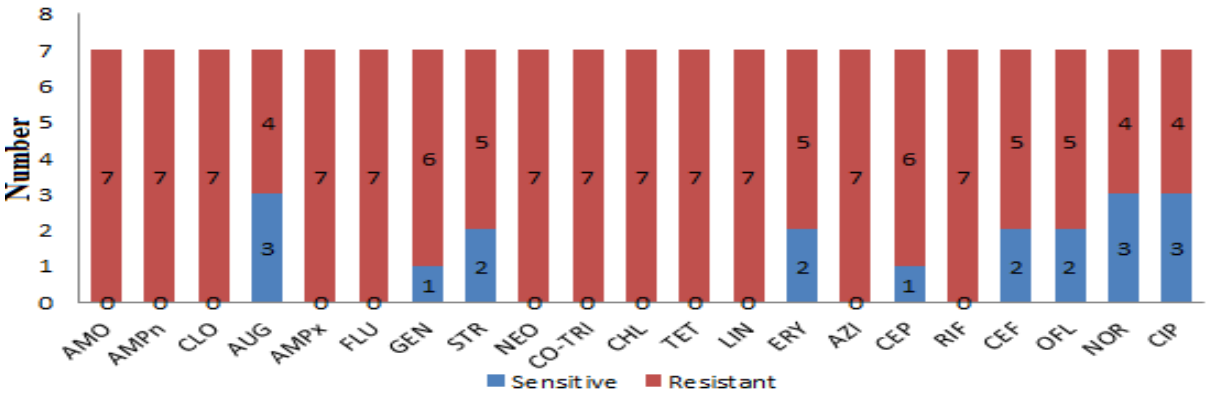

Figure 4: The antibiogram types of *staphylococcus saprophyticus* isolated from female urine samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN=Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxcine, CIP=Ciprofloxacin

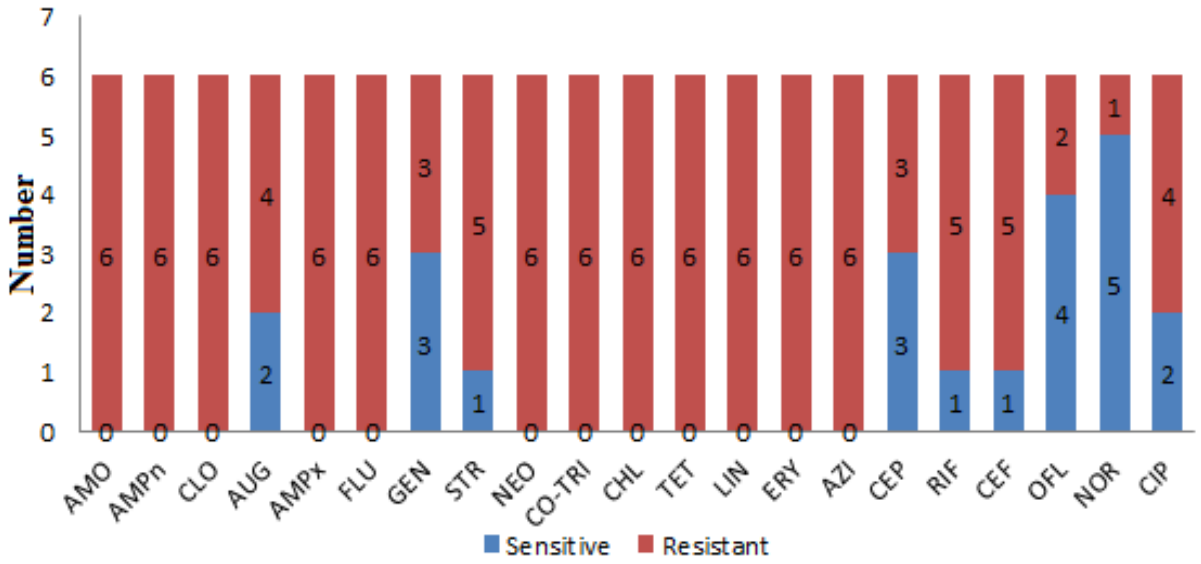

Figure 5: The antibiogram types of *staphylococcus epidermidis* isolated from female urine samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN=Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxcine, CIP=Ciprofloxacin

DISCUSSION

UTI is the most common health care associated group of bacterial infections affecting humans in Africa.[26] It is the major cause of morbidity in both the hospital and community settings[27] and affecting both out and in patients.[28] The observed 186 cases of UTI of staphylococcus spp infection between January and December in the study area indicate the challenging nature of Straphylococcus infection. In this study, S. aureus and S. saprophyticus were more prevalence in male urine samples compared to the female urine sample; where S. epidermidis was more prevalent. In line with our study, similar higher prevalence of S. aureus in male than female has been documented in Otajevwo[29] when male presented 19.1% as

compared to 6.7% in female. The reason for the high prevalence of *Staphylococcus aureus* in males is not clear, but according to Orrett and Davis[30], lack of circumcision, receptive *anal* intercourse and HIV infection are recognized risk factors for UTI in males.

Studies of the antibiogram pattern of *Staphylococcus* spp. are very useful and provide data that assist with strategies for avoiding the dispersion of antibiotic resistant especially the multidrug-resistant (MDR) that has become a public health issue. In this study for example, S. aureus from male and female urine samples were sensitive to the 21 antibiotics to different degree, however, no antibiotic reported 50% sensitivity. For S. aureus from male urine samples, augmentin and azithromycin were the most sensitive with only 28/92 (30.43%) and 19/92 (20.65%) of the isolates showing susceptibility. On the other hand, noreloxcine and gentamizin were the most sensitive with 26/72 (36.11%) and 20/72 (27.78%) of the S. aureus isolates from female urine samples showing susceptibility. Similar to these findings, Akortha and Ibadin[31] have documented 83% and 50.2% susceptibility to augmentin and gentamicin respectively in UTIs *S. aureus* in Benin City, Nigeria. The least sensitivity (less that 10% susceptibility), were observed with ampicillin, ampiclox, streptomycin, neomycin, co-trimoxazole, chloramphenicol, tetracycline and ciprofloxacin for the male urine samples while for the female Amoxicillin, Ampicillin, Cloxacilin, Ampiclox, Flucloxacilin, Neomtcin, Co-trimoxazole, Chloramphenicol, Tetracycline, Lincocin, Erythromycin and Azithromycin were the least sensitive. In a study to determined the antimicrobial resistance profile of *S. aureus* strains from patients with UTI in Yenagoa, South-South, Nigeria, Onanuga and Awhowho[32] reported *S. aureus* to showed 100% resistance to ampicillin and cefoxitin, 97.8% to tetracycline and 80.4% to chloramphenicol and co-trimoxazole.

For *S. saprophyticus* from male urine samples, Ofloxacin was the most sensitive (6/9; 66.67%) while Cefuroxime, Noreloxcine, and Ciprofloxacin were 44.44% sensitive. On the other hand, Augmentin, Noreloxcine, and Ciprofloxacin were the most sensitive with 42.86% subseceptibility each for S. saprophyticus isolated from female urine sample. Khoshbakht *et al*.[33] in a study of bacterial strains isolated from urinary tract infections in Karaj, Iran showed that nitrofurantoin and vancomycin are highly susceptibility (92.3%) to *S. saprophyticus* isolates. In this study, *S. saprophyticus* from male urine samples was 100% resistant to Amoxicillin, Ampicillin, Cloxacilin, Ampiclox, Flucloxacilin, Neomycin, Co-trimoxazole, Chloramphenicol, Tetracycline and Lincocin and that from female urine sample was 100% resistant to Amoxicillin, Ampicillin, Cloxacilin,, Ampiclox, Flucloxacilin

Neomycin, Co-trimoxazole, Chloramphenicol, Tetracycline, Lincocin and Rifampicin. These findings indicate that male and female S. saprophyticus are multi-drug resistant. In agreement to our findings, Khoshbakht *et al*.[33] has also documented *S. saprophyticus* isolates to exhibited high resistance to Ampicillin, Tetracycline and Erythromycin (92.31%).

On *staphylococcus epidermidis* isolated from female urine samples, Noreloxcine was the most sensitive antibiotic with susceptibility of 83.33% and this was followed by Ofloxacin with susceptibility of 66.67% and Gentamycin and Cephalexin with susceptibility of 50.0% each. In a study by Mohammad et al.[34], vancomycin (86.1%) and gentamicin (74.0%) were reported as most effective antibiotics against *S. epidermidis*. However, S. epidermidis was 100% resistant to Amoxicillin, Ampicillin, Cloxacilin, Ampiclox, Flucloxacilin, Neomtcin, Co-trimoxazole, Chloramphenicol, Tetracycline, Lincocin, Erythromycin and Azithromycin. In line with the findings of this study, *S. epidermidis* has been showed to be highly resistance to methicillin (84.1%) and penicillin (94.2%) and it is estimated that amikacin and ceftriaxone lose their efficiency on these bacteria soon.[34]

Ako-Nai et al.[35] presented a report in which 67.3% of staphylococcal isolates were resistant to cloxacillin, 64.9% resistant to amoxicillin, 51.8% to augmentin, 70.2% to tetracycline, 48.8% to erythromycin, 36.9% to cotrimoxazole, 11.9% to chloramphenicol and 1.8% to gentamycin in a study Ibadan, Nigeria. The high resistance of the bacteria to commonly available and used antibiotics observed in this study is worrisome considering that they are easily available in the community without prescription and antimicrobial therapy with them are most times initiated even before the seeking medical help or having urine culture. There are many reasons for this alarming phenomenon, which according to Tolun et al.[36] including inappropriate prescribing of antibiotics and poor infection control strategies. The 100% resistivity of S. saprophyticus and S. *epidermidis* isolates to 10 to 12 different antibiotics by both male and female isolates, indicate the presence of strong selective pressures from the antibiotics in the community in this study. By implication, this study strongly shows the need for *in-vitro* antibiotic susceptibility pattern before initiation of antibiotic therapy.

Conclusively, the findings of this study indicate that gender affect *Staphylococcus* spp. susceptibility pattern to antibiotics and that multi-drug resistant within the study area is high; especially with the common antibiotics. Judging from the findings of this study, augmentin and azithromycin, noreloxcine and gentamizin, Ofloxacin, Cefuroxime, Noreloxcine, and Ciprofloxacin are considered as appropriate antimicrobials for empirical treatment of UTI of

staphylococcus origin in the area. However, there are potency variations in these antibiotics with gender. The different in susceptibility patterns in male and female and to different antibiotics in this study suggest the extremely need for sensitivity assessment of individual prior to antibiotic therapy in UTIs patients.

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