ABSTRACT

An infection of any kind attacking any part of the urinary system is termed Urinary Tract Infection (UTI) which has continued to be a serious healthcare challenge globally. This research work therefore, was aimed at determining the prevalence and antibiotic resistance profile of Staphylococcus aureus and Escherichia coli among patients attending Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria. These two microorganisms of interest were isolated and identified from midstream urine samples of 202 patients suspected of having UTIs through the use of microbiological techniques (culture, microscopy, and biochemical tests). Antimicrobial Susceptibility Testing of the isolates was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI). The prevalence of UTI cases were found to be 51.49% of the total samples analyzed. The Staphylococcus aureus had high prevalence of 59.62% compared to E. coli, which had a prevalence of 40.38%. The findings revealed that UTIs are more prevalent among males than in females. The highest prevalence of UTIs was found within the age group of 30-39 years (59.62%) followed by patients within the age group of 70 years and above with prevalence rate of 26.92%. E. coli had the lowest mean average (78.35%) of resistance compared to Staphylococcus aureus which had the highest (80.77%). Resistance to antibiotics ranges from 64.29% (Levofloxacin) to 89% (Nalidixic acid) for E. coli and 74.19% (Levofloxacin) to 93.55% (Rifampicin) for S. aureus. This high rate of resistance could be associated with self-prescription, incomplete dosage and indiscriminate use of antibiotics. Bacteria strains resistance to multiple antibiotics exists among patients suffering from UTIs.

Keywords: Staphylococcus aureus, Escherichia coli, Urine samples, Antibiotic resistance, UTI.

INTRODUCTION

An infection of any kind attacking any part of the urinary system is termed Urinary Tract Infections (UTIs) (Anejo-Okopi et al., 2015; Derbie et al., 2017; Sule et al., 2018). UTIs have continued to be a serious healthcare challenge, affecting over 250 million individuals globally each year (Derbie et al., 2017; Sule et al., 2018). This is attributed to the ability of the pathogenic microorganism to successfully colonize the urinary tract and cause a disease condition (Otajewo, 2013). The microorganisms often associated with UTIs include; bacteria, fungi, yeast or protozoa, virus or even parasites (Ajao and Yakubu, 2015). However, bacteria have been reported as the major uropathogen affecting the urinary tract (Khoshbakht et al., 2013). These infections are also reported to be the cause of most hospital visits and the most associated nosocomial infection of the humans (Timothy et al., 2014; Sule et al., 2018).

Epidemiologically, uropathogenic infections are in the increase with associated cause of most morbidity and mortality worldwide (Timothy et al., 2014; Tahira et al., 2020). It is estimated that over 250 million people worldwide are infected with UTI yearly; which are mostly caused during hospital visits (Sule et al., 2018; Tahira et al., 2020), which is seen to be a burden to the nations and individuals affected (Akingbade et al., 2014). The prevalence of UTI in Nigeria varies across geo-political zones. Among the semi-urban settlers in South-West Nigeria, the prevalence rate was reported to be 11.96%, 2.7% prevalence in South-East urban settlers, 13.7% prevalence in North-East (Akinsote et al., 2018), 35.5% North-Central Nigeria (Muhammed, 2015).
Pathogenic UTIs cases is drastically changing with several etiologic agents of UTIs been reported (Alo et al., 2015). Gram-positive organisms like *Staphylococcus aureus* and *Streptococcus pyogenes* and other Gram-negative species such as *Escherichia coli* are the predominantly reported organisms (Sule et al., 2018), others include; *Klebsiella pneumonia* and *Proteus mirabilis* (Reis et al., 2016). *Staphylococcus aureus* and *Escherichia coli* are documented to be the leading cause of human and animal infections, causing variety of infections; from the squamous and tissue infections, soft tissue infections, surgical site infection, bone infections, joint infections, and urinary tract infections to enteric and systemic infections. *Staphylococcus aureus* is known to be the commonest cause of hospital-acquired infection and bacteremia. It has also been established to be the cause of most hospital acquired respiratory infections (Onanuga and Awhowho, 2012). Other pathogens such as *Klebsiella* species, *Enterobacter* species, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Citrobacter sp.*, *Staphylococcus sp.*, *Neisseria gonorrhoea* and *Candida sp.* are reported to be the cause of most UTIs (Timothy et al., 2014; Ekwealor et al., 2016; Derbie et al., 2017; Tahira et al., 2020). However, some previous studies have reported the increasing prevalence of *S. aureus* in UTIs (Onanuga and Awhowho, 2012).

The predisposing factors to UTIs includes: sexual intercourse with infected person (Tahira et al., 2020). In relation to gender, females are most predisposed (Tahira et al., 2020). Other predisposing factors include inheritance, disease condition such as diabetes, stoppage of menses, poor nutrition, urination after sex, kidney stones, old age, utilization of urinary catheter, pregnancy, prostrate syndrome among males, diaphragm use, abnormalities in the urinary tract as well as poor hygiene practices (Dash et al., 2013; Anejo-Okopi et al., 2015; Ekwealor et al., 2016; Tahira et al., 2020). Also, previous exposure to UTIs is known to be a predisposing factor (Moges et al., 2003). Antibiotic resistance (AR) by microorganisms has become a major threat to public health with more microorganisms becoming resistant multiple antibiotics (Ochada et al., 2015). This resistance could be natural, acquired or clinical and can be defined as the ability of microorganisms such as pathogenic bacteria to resist the effect of antibiotic or antimicrobial agents (Nolte, 2014). The natural or intrinsic resistance is an inborn caution for bacteria to resist antibiotics without previously exposed to antibiotic or without horizontal gene transfer while the acquired resistance is cause by intrinsic gene mutation before exposure to some mutagens, antibiotic or through horizontal genetic transfer (Hollenbeck and Rice, 2012). Diagnosis of UTIs by doctors and physicians in the out-patient department is principally through the presentation of signs and symptoms without laboratory test result obtained; because an immediate treatment is needed to be administered. Laboratory diagnosis on the other hand is based on urinalysis, dipstick leukocyte esterase test for detecting blood in urine (pyuria) with the ability to detect 10 white blood cells in millimeter cube of urine sample (10 WBC/mm³) (Sobel, 2014). The presence of Nitrite in a urine sample is an indication of UTIs (Helen and Jennifer, 2018). However, urine microscopy, culture and sensitivity (M/C/S) are the widely used and most preferred methods. Treatment of UTIs is mainly by the administration of antibiotics through various means; cutaneous, venous or muscally.

In Nigeria, most UTIs cases in symptomatic patients usually indulged in indiscriminate use of antibiotics before consulting the physicians or clinicians, when they could no longer treat or control the symptomatic conditions. On the other hand, the physicians usually prescribe and treat the patients with wide broad-spectrum antibiotics without any microbiological findings (Aboderin et al., 2009; Oli et al., 2017). These deep indiscriminate use and inappropriate prescription of antibiotics in the treatment of UTIs are vital contributing factors to the emergence and widespread of bacterial resistance to the most commonly used antimicrobial agents (Sule et al., 2018). The situation is deteriorating with the increase prevalence of substandard and fake antimicrobial agents marketed in the country (Nigeria) (Oli et al., 2017). Antibiotic resistant pathogens have degraded the choices of administering an effective antibiotic treatment (Cassir et al., 2014; Moroh et al., 2014). Furthermore, as the number of resistant strains increase in clinical environment, broad spectrum antibiotics becomes the paramount choice, but the incorporation of resistance to broad spectrum antibiotics in multidrug resistant strains degrades the chances of selecting an effective empirical treatment (Hirsch and Tam, 2010). While the prevailing crisis of AR has been reported elsewhere, the resistance status towards commonly used antibiotics at Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria is not yet known. There is a limited report of AR in Lafia, Nasarawa State, Nigeria on commonly used antibiotics in the hospitals. The changing spectrum of microorganisms involved in UTIs necessitates...
the need for continuous and regular antibiotic resistance (AR) surveillance of these organisms in order to guide empirical treatment in UTIs. This cross-sectional designed research work was aimed at determining the prevalence and AR profiles of _S. aureus_ and _E. coli_ strains from patients with UTIs attending Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria.

**MATERIALS AND METHODS**

**Study Location**
The study was conducted among patients attending Urology Clinic in Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria. Lafia is the state capital of Nasarawa state situated in the North central part of Nigeria lying at latitude 8°29’30” North of the equator and longitude 8°31’0” East of Greenwich Meridian (Akwa et al., 2007). Lafia has a total inhabitant of 330, 712 (Census, 2006; Akwa et al., 2007). It is currently reported that Lafia has a population of 127, 236 (WPR, 2019).

**Ethical Approval**
Ethical approval for the study was secured from Dalhatu Araf Specialist Hospital ethical committee, in accordance with the code of ethics for biomedical research involving human subjects. The subjects were recruited after they were given health talk on the aim and importance of the study.

**Consent**
The participants were consented and a form was given to them to fill and sign in other for them to participate fully in this study. A questionnaire was used to obtained demographic data of participants such as age, sex, use of catheter as well as risk factors. The information obtained from the participants was assured to be confidential and kept out of third-party disposal.

A total of approximately 194 samples were calculated based on prevalence rate of 14.8%. However, 202 patients were selected for the study to allow for non-response.

**Sample Collection**
A total of 202 urine samples were collected from patients attending Urology Clinic of the hospital between July 2019 and September 2019. The recruitment of the study patients was randomized, as only patients attending the clinic were used irrespective of their complaints or whether they have symptoms associated with UTIs. The patients were told how to collect urine sample. Mid-stream urine specimens were collected from each of the patient between 6a.m. and 9a.m. into sterile bottles. Their caps were tightly closed then stored in iced packed containers for onward transport to the laboratory. To avoid cross-contamination during sampling, sterile gloves and forceps were used. The samples were immediately transported to the Microbiology laboratory of Federal of University, Lafia for culture and other microbiological analysis.

**Isolation and Identification of Test Organisms**
Isolation of _S. aureus_ and _E. coli_ were carried out with the aid of a standard wire loop (0.001 ml), where the urine from the sample container were aseptically streaked onto Mannitol Salt Agar (MSA) and Eosin Methylene Blue Agar (EMBA) media plates and incubated at 37°C for 24hrs as described by Cheesbrough, (2006). Greenish metallic sheen colonies that grew on EMBA were selected as presumptive _E. coli_ while those appearing yellow with color change on MSA plates were selected as presumptive for _S. aureus_. The presumptive isolates were identified through microscopy (Gram stain) and minimum selective biochemical tests notably catalase, coagulase, indole and citrate tests respectively. The isolates were then stored in a nutrient agar slant for subsequent analysis.

**Antibiotic Profile:**
The antibiotic resistance profile test for _S. aureus_ and _E. coli_ were carried out as prescribed by Sule et al. (2018) following standard protocols laid by Clinical and Laboratory Standard Institute (CLSI), (2014). The pure isolates were standardized to turbidity of 0.5 McFarland standards before surface inoculation on Muller Hinton agar (MHA) using a sterile swab stick. The inoculated plates were allowed for 15 minutes to ensure the surface of the culture plate is dried and ready for antimicrobial testing. Antimicrobial discs of known concentration were placed on the surface of the inoculated MHA respectively (i.e. Gram-negative _E. coli_ and Gram-positive _S. aureus_) using sterile forceps. The plates were then incubated in the incubator for 37°C for 18hrs, after which the zone of inhibition was measured using a metre rule. The antibiotics discs used include; Ofloxacin (10µg), Levofloxacin (10µg), Nalidixic acid (30µg), Ciprofloxacin (10µg), Augmentin (30µg), Gentamicin (10µg), Cotrimoxazole (30µg), Streptomycin (30µg), Cefalexin (10µg), and Ampicillin (30µg) while Gram positive disc contained Ciprofloxacin (10µg), Norfloxacain (10µg), Gentamycin (10µg), Streptomycin (30µg), Amoxicill in (20µg), Erythromycin (30µg), Rifampicin (20µg), Chloramphenicol (30µg), Ampicillin (20µg), and Levofloxacin (20µg) (Godwin, et al., 2018).
Statistical Analysis
The data obtained in this research work were subjected to simple statistical analysis and analysis of variance (ANOVA). A p-value of less than or equal to 0.05 is considered to be statistically significant (P ≤ 0.05).

RESULTS
Of all of the 202 patients recruited for this study completed their questionnaire correctly giving a response rate of 100%, this was achieved through the entry point used in administering the questionnaires. There were 153 (75.74%) males and 49 (24.26%) females. Out of which 88 (84.62%) and 16 (15.38%) male and female have significant bacteriuria respectively (Table 1). The use of catheter was observed in 65.35% participants with 67.31% having significant bacteriuria. The antimicrobial profile of \textit{S. aureus} and \textit{E. coli} from the result of this research work (Table 2), showed that Levofloxacin had the highest rate of sensitivity against both \textit{S. aureus} (74.19%) and \textit{E. coli} (64.29%). However, \textit{S. aureus} was highly resistant (93.55%) to Rifampicin whereas \textit{E. coli} was most resistant (89.00%) to Nalidixic acid.

Table 1: Socio-demographic characteristics and Anthropogenic factors associated with Urinary Tract Infections.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>No. of Samples Collected (n =202)</th>
<th>Samples with Sig. Bacteriuria</th>
<th>% of Sig. Bacteriuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 29</td>
<td>5</td>
<td>2</td>
<td>1.92</td>
</tr>
<tr>
<td>30 – 39</td>
<td>35</td>
<td>60</td>
<td>57.69</td>
</tr>
<tr>
<td>40 – 49</td>
<td>10</td>
<td>6</td>
<td>5.77</td>
</tr>
<tr>
<td>50 – 59</td>
<td>21</td>
<td>5</td>
<td>4.81</td>
</tr>
<tr>
<td>60 – 69</td>
<td>55</td>
<td>3</td>
<td>2.88</td>
</tr>
<tr>
<td>≥70</td>
<td>76</td>
<td>28</td>
<td>26.92</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>153</td>
<td>88</td>
<td>84.62</td>
</tr>
<tr>
<td>Female</td>
<td>49</td>
<td>16</td>
<td>15.38</td>
</tr>
<tr>
<td>Use of catheter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>132</td>
<td>70</td>
<td>67.31</td>
</tr>
<tr>
<td>No</td>
<td>70</td>
<td>34</td>
<td>32.69</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic admin.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>11</td>
<td>8</td>
<td>7.69</td>
</tr>
<tr>
<td>1 week before</td>
<td>21</td>
<td>16</td>
<td>15.38</td>
</tr>
<tr>
<td>2 weeks before</td>
<td>32</td>
<td>20</td>
<td>19.23</td>
</tr>
<tr>
<td>1 month before</td>
<td>50</td>
<td>40</td>
<td>38.47</td>
</tr>
<tr>
<td>Not on drugs</td>
<td>88</td>
<td>20</td>
<td>19.23</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>104</td>
<td>100</td>
</tr>
</tbody>
</table>

N= total number of samples, No.= number, yrs= years, admin= administration, Sig.= Significant.
**DISCUSSION**

Urinary tract infections (UTIs) have been documented to be caused mostly by Gram-negative enterobacteria with *E. coli* being the most prevalent (Onanuga and Awhowo, 2012; Moroh et al., 2014; Tahira et al., 2020). However, there is an increasing prevalence of *S. aureus* as a UTIs etiologic agent with a fearful rate of developing antimicrobial resistance (Onanuga and Awhowo, 2012). Resistance empowers bacteria to escape the attack by antibiotics and in turn slows the ability to treat infections (Spellberg et al., 2008). Thus, antibiotics resistance has been considered one of the vital threats to medicine (Walker et al., 2009).

Urinary Tract Infection is a major cause of morbidity and mortality. Among a total number of 202 patients analyzed in this study, the prevalence of 51.49% positive isolates was recorded. This is closely related to a previous study carried out by Otajevwo and Amedu, (2015) which they recorded a prevalence rate of 59.2% in Evbuobanosa, Edo State. However, this prevalence was more than those reported by Iregbu and Nwajiobi, (2013) and Aiyegoro et al. (2007) in Abuja and Ile-Ife both in Nigeria which they recorded a prevalence of 13% and 11.9% respectively. The variations established in the studies could be attributed to variations in study populations and/or in the criteria employed by various centers in selecting urine samples for culture.

The prevalence of *S. aureus* in this study was 62 (30.69%) which is not far from that reported by Martin et al. (2019) while that of *E. coli* recorded was 42 (20.79) which shows a great variation with the work conducted by Martin et al. (2019) who reported a prevalence rate of 36/86 (41.9%) and also the work reported by Mayanga et al. (2005) who reported an estimated prevalence range between 40 to 46% of *E. coli*. The infection with *S. aureus* indicates contamination during instrumentation and also possibly due to contamination with the normal flora of the hands (Iregbu and Nwajiobi, 2013). The findings in this research revealed that UTIs are more prevalent among males compared to their female counterparts. This higher prevalence in males may be attributed to the fact that more males attend the clinic than females as well as inadequate awareness of UTIs with emphasis on the danger it carries to them and the general public. This is in contrast with reports of Derbie et al. (2017) in which they started the nature of female anatomy being a major factor contributing to the high prevalence in females than their male counterparts. However, the finding in this research was in accordance with that reported by Otajevwo and Amedu, (2015). In their study, they reported a prevalence rate of 57.1% (compared to 51.49% obtained in this research work) in males without stating clearly the reason for that. However, this may be attributed to lack of circumcision, receptive anal intercourse and HIV infection among the study participants.

### Table 2: Antibiotic resistance profile of *Staphylococcus aureus* and *Escherichia coli* (N = 62; n = 42)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Disk content (µg)</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPR 10</td>
<td>13</td>
<td>13(79.03)</td>
<td>13(69.04)</td>
</tr>
<tr>
<td>LEV 20</td>
<td>5</td>
<td>5(74.19)</td>
<td>15(64.29)</td>
</tr>
<tr>
<td>RD 20</td>
<td>4</td>
<td>4(93.55)</td>
<td></td>
</tr>
<tr>
<td>S 30</td>
<td>10</td>
<td>10(88.29)</td>
<td>6(85.71)</td>
</tr>
<tr>
<td>NB 10</td>
<td>14</td>
<td>14(77.42)</td>
<td></td>
</tr>
<tr>
<td>ERY 30</td>
<td>8</td>
<td>8(88.29)</td>
<td>6(85.71)</td>
</tr>
<tr>
<td>CN 10</td>
<td>13</td>
<td>13(79.03)</td>
<td></td>
</tr>
<tr>
<td>CH 30</td>
<td>18</td>
<td>18(70.97)</td>
<td></td>
</tr>
<tr>
<td>APX 20</td>
<td>1</td>
<td>1(88.29)</td>
<td></td>
</tr>
<tr>
<td>AMX 30</td>
<td>16</td>
<td>16(74.20)</td>
<td></td>
</tr>
<tr>
<td>OFL 10</td>
<td>12</td>
<td>12(71.43)</td>
<td></td>
</tr>
<tr>
<td>NA 30</td>
<td>11</td>
<td>11(89.00)</td>
<td></td>
</tr>
<tr>
<td>AUG 30</td>
<td>6</td>
<td>6(85.71)</td>
<td></td>
</tr>
<tr>
<td>SXT 30</td>
<td>10</td>
<td>10(76.19)</td>
<td></td>
</tr>
<tr>
<td>CEP 10</td>
<td>1</td>
<td>11(89)</td>
<td></td>
</tr>
<tr>
<td>PN 30</td>
<td>14</td>
<td>14(66.67)</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** N = number of positive *S. aureus*, n = number of positive *E. coli*, CPR = ciprofloxacin, LEV = levofloxacin, RD = rifampicin, S = streptomycin, NB = norfloxacin, ERY = erythromycin, CN = gentamycin, CH = chloramphenicol, APX = ampicillin, AMX = amoxicillin, SXT = cotrimoxazol, PN = ampicillin, OFL = ofloxacin, CEP = cefalexin, NA = nalidixic acid, AUG = augmentin.
CONCLUSION

There is the emergence of multiple drug resistance of S. aureus and E. coli among patients visiting DASH Lafia. Their prevalence was high among male patients compared to their female counterpart and the occurrence was more among patients within the age group of 30-39 years. This high rate of resistance could be associated with self-prescription, incomplete dosage and indiscriminate use of antibiotics. Bacteria strains resistance to multiple antibiotics exists among patients suffering from UTIs.

Limitation and Recommendation

The findings in this study are only limited to two hundred and two (202) samples, a national antibiotic resistance surveillance of these isolates is recommended for further study. Public awareness should be increased on the impacts caused by indiscriminate use and self-prescription of antibiotics. Development and strict adherence to antibiotic policy should be implemented to guide the sale, prescription and use of antibiotics by the masses and healthcare workers.

Contributions of Authors

This work was carried out in collaboration between all authors. Authors ASR and FNJ designed the study, help in sample collection, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MCO and YY managed the analyses of the study. Authors ASR and YY managed the literature searches and wrote the final manuscript. Author CA proofread and approved the final manuscript.

Competing Interests

Authors have declared no competing interests.

Acknowledgement

We are sincerely grateful to the management, lecturers and technologists of the department of Microbiology, Federal University of Lafia. Profound gratitude also goes to Dalhatu Araf Specialist Hospital ethical committee for the approval of their health institutions as center for this study. We also appreciate all the staff of the Urology department of this health center for their cooperation and technical supports in the collection of specimens from the patients.

REFERENCES


