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PREVALENCE OF UROPATHOGENIC *ESCHERICHIA COLI* & *STAPHYLOCOCCUS AUREUS* AMONG PATIENTS WITH CASES OF URINARY TRACT INFECTIONS ATTENDING BENUE STATE UNIVERSITY TEACHING HOSPITAL, MAKURDI

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ABSTRACT
In the present study, we investigated the prevalence of few bacterial organisms associated with urinary tracts infection and their antimicrobial susceptibility patterns. Total of Ninety (90) midstream urine samples were collected from outpatients with respect to their age group, gender and marital status attending health facility in Benue State University Teaching Hospital Makurdi. Each 0.1ml of urine sample was cultured on MacConkey agar and colonies showing lactose fermentation were subsequently subcultured on Eosin Methylene Blue Agar to obtain a pure culture of *E. coli* whereas *S. aureus* was isolated by inoculating 0.1 ml of urine sample on Cysteine Lactose Electron Deficient (CLED) agar and subsequently subcultured into Manitol Salt Agar to obtain pure culture. One way analysis of variance (ANOVA) and paired t-test was carried out using IBM SPSS STATISTICAL version 22. Based on the result obtained, 33(36.67%) prevalence of *E. coli* and *S. aureus* was observed. *E. coli* has the highest occurrence of 19(95.00)% while *S. aureus* has 14(70.00%). There was significant variation within the age group at $\rho<0.05$ with the highest 11.11% occurring in 27-32 years while the lowest was 4.44% in 33-38 and 39-44years respectively. On the gender basis, female had highest occurrence of 21.11% while male has the lowest of 15.56%. Variation in the positive samples on marital status showed significant difference with highest among married subjects 21.11% while the lowest occurred in single 15.56% at $\rho<0.05$. *E. Coli* was sensitive to Ofloxacin (15.00mm), Ciprofloxacin (12.00mm) and Prefloxacin (12.22mm). and resistant to amoxicilin and ampicilin while *S. aureus* was sensitive to amoxicilin (14.44), ofloxacion (12.22mm) and ciprofloxacin (12.22mm) but resistant to vancomycin and prefloxacin respectively. Based on this study, many isolates were resistant to the antimicrobial agents. This therefore recommended recommends antimicrobial sensitivity assay to know the most effective drugs before administration of any drug for UTIs.

KEYWORDS: Prevalence, *E. coli*, *S. aureus*, Outpatients, Urinary Tract Infection.

INTRODUCTION
Urinary tract infections (UTIs) also known as acute cystis or bladder infections are a serious health problem affecting millions of people globally in each year and are the most common bacterial infections in patient of all ages with high risk in young women resulting in significant and health care cost. The infection affects more female than male with at least one-fifth of them infected at a point during their lifetime though some infections in women are symptomatic (Mims *et al.*, 1998, Mullenix and Prince., 1999). According to Kunin (2011), Urinary tract infections (UTIs) are one of the most common microbial diseases encountered in medical practice affecting people of all ages. Worldwide, UTIs' prevalence was estimated to be around 150 million persons per year (Gupta, 2001). This infection is predominantly caused by bacteria, even though some strains of viruses (adenovirus), fungi (Candida albican) and protozoa (*Trichomonas vaginalis*) can infect the urinary tract (Abdulamir and Hafidh 2009). Research estimated that over 85% of UTIs are caused by bacteria from intestine or vagina (Abubakar *et al., 2009*). Bacterial infection of the lower urinary tract (bladder) and urethra are very common especially in young, sexually active women (*Caraccilo et al., 2011*). Common clinical symptoms associated with UTI that brings medical attention are those referable to the urinary includes; Dysuria – its early symptoms, may be burning or pain on the tip of the penis (for men), itching or in pain during urination, discomfort in the lower abdomen

and a frequent urge to urinate may arise (Stamm, 1997). According to Adeleke & Olarinde (2013), E*scherichia coli* is the most commonly cause of UTIs in Gram-negative bacteria among all bacteria associated to UTIs. Other Gram negative bacteria includes: *Pseudomonas aeruginosa* and *Acinetobacter* spp. while associated Gram-positive bacteria are *Enterococcus faecalis*, capnophilic corynebacteria and lactobacilli, and staphylococci, which play minimal roles, have now assumed significant places as etiological agents of UTIs (Mims *et al*., 1998, Mbata, 2007).

Tambekart et al. (2009), reported that Uropathogenic *Escherichia coli* (UPEC) is one of the main causes of UTIs while serving as part of normal flora in the gut and can be introduced in many ways. In particular for females, the directions of wiping after defecation (wiping back to front) can lead to faecal contamination of the uroginetal orifices alongside anal sex can also introduce these bacteria into the male urethra, and in switching from the anal to vaginal intercourse the male can introduce UPEC to the female urogenital system as well *(Macejko et al., 2007).*

(Kluytmas *et al* 2001). *S. aureus* is the most common species of *staphylococcus* to cause *Staphylococcus* infections and is a successful pathogen due to a combination of nasal carriage and bacterial immune-evasive strategies. (Kluytmas *et al.,* 2001). *S. aureus* can cause a range of illness, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, oteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. It incidence range from skin soft tissue, respiratory, bone, joint, endovacuar to wound infections. It is still one of the five most common causes of nosocomial infection and is often the cause of post surgical wound infections.
Antimicrobial resistance has become a significant problem worldwide) as bacterial resistance to antimicrobial agents has become emerging and rapidly disseminating among many nosocomial and community –acquired pathogens *(Gul et al., 2017).* The development of antibiotic resistance in *E. coli* has important clinical implication and the development of resistance to older antimicrobial agent such as ampicillin and trimethptrim-sulfamethoxazole as well as the emerging problem of fluoroquinolone resistance may substantially limit our antibiotic choices *(Manaal et al., 2011).*

Grabe *et al.* (2015), documented that resistance of organisms to some antibiotics has increased and it has been observed that frequent use of these antibiotics have caused an visible increase in the development of resistance, third generation Cephalosporin's with aminoglycosides, Quoninoles are used to treat acute UTIs. Recent studies showed that patient with suspected cases of UTIs are known to undergo self-medication which leads to antibacterial resistance.

In spite of the preceding and the continuous scrutiny of improved pathogenicity of microorganisms and their associated resistance to antimicrobial agents especially in regards to urinary tract infections (UTIs), relatively few documented report on its incidence and antimicrobial resistance via microbial production are available in Nigeria. This study therefore, was done to evaluate the prevalence of UTIs among outpatients attending Benue State University Teaching Hospital Makurdi and to proffer promising recommendation based on the findings.

METHODOLOGY

Collection of sample

A total of ninety (90) midstream urine sample was collected from outpatients attending health care facility in Benue State University Teaching Hospital Makurdi (BSUTH) using sterile centrifuge tube and immediately transported to the laboratory for analysis.

Isolation of *Escherichia coli*

The urine sample was cultured on MacConkey agar (MCA) and colonies showing lactose fermentation were subsequently subcultured on Eosin Methylene Blue (EMB) Agar to obtain a pure culture of *Escherichia coli*.

Isolation of *Staphylococcus aureus*

For *Staphylococcus aureus*, 0.1 ml of each urine sample was inoculated on Cysteine Lactose Electron Deficient (CLED) agar using spread plate technique. It was allowed to stand for five (5) minutes and incubated inversely for 24 hours at 37^0C. Colonies exhibited golden colours were subcultured into Manitol Salt Agar (MSA).

Biochemical examination

Biochemical examination of the presumptive organisms was carried out according to method described by Cheesbrough (2006) with modification.

Gram Stain

A small portion of each presumptive isolate was emulsified in a drop of water on a clean grease-free glass slide; heat fixed and allowed to air dried. The fixed smear was flooded with crystal violet for 30-60 seconds and rapidly rinsed off with clean water, with the water appropriately tipper off. The smear was then covered with Gram's iodine for 30-60 seconds and rinsed with water. Rapid decolourization of the smear was done for a few seconds using acetone-alcohol and immediately rinsed with clean water. Counter staining was done for 60 seconds using safranin and subsequently rinsed with clean water. The back of the slide was cleaned and the slide was allowed to air dry. After air drying, the smear was viewed under the microscope using the oil immersion (×100) objective**.**

Coagulase test

The slide test method of detecting the enzyme coagulase was used. A drop of rabbit plasma were placed on a clean grease free slide and 3-4 pure colonies were

emulsified in a drop of plasma and observed for 3-5 minutes. Agglutination of the cells on the slide within one to two minutes indicated the presence of coagulase.

Indole test: To perform this test, 3 ml of sterile tryptone water was dispensed into a sterile bijou bottle, inoculated with the test organisms and incubated for 48 hours at 35°C. After incubation, 0.5 ml of Kovac's reagent was added to the bijou bottle and agitated gently. Formation of red coloration on the surface of the mixture within 10 minutes signified a positive result (Anzaku & Pedro, 2017).

Motility Test

The motility medium was inoculated making a fine stab with a needle to a depth of about 1-2cm short of the bottom of the tube. It was then incubated at 37^{o}C for 24 to 48 hours and the tube was examined at the end of the period of incubation. A positive result was indicated by the absence of a well-defined line of growth and the presence of cloudiness within the medium while negative result was indicated by restricted growth to the line of inoculation.

Antimicrobial susceptibility assay

Antimicrobial susceptibility test was carried out in each of the plate using Bauer-Kirby agar disc diffusion method (Anzaku *et al.*, 2017).

The antibiotics tested include: Vancomycin, amoxacilin ampicilin prefloxacin and ciprofloxacin accordingly.

Three to five well isolated colonies of *E. coli* and *S. aureus* were transferred into 5 mL Nutrient broth and incubated at 37°C for 18 to 24 h. The overnight broth culture was, diluted using sterile distilled water to a turbidity equivalent to 0.5 McFarland standard (approximately 10^8 cfu/ml), and inoculated onto the entire surface of a dried Mueller-Hinton Agar (MHA, Oxoid) plate creating a lawn of the culture. The surfaces of the inoculated MHA plates were allowed to dry at room temperature before placing the antibiotic discs followed by incubation. After incubation for 24 h at 37°C, the diameter (in mm) of the inhibition zone around each disk was measured.

RESULTS AND DISCUSSION

4.1 Occurrence of E. coli and *S. aureus* isolates with respect to age group.

Occurrence of *E. coli* and *S. aureus* isolates is shown in Table 1 below with the total percentage occurrence of 33(36.67%). Highest positive occurrence of 11.11% was obtained in the age group of 27-32years age range of 21-26 had 7.78% followed 6.67% occurring in the age groups of 15-20 and o33-38 and 39-44years. The least occurrence was observed in the age groups of 39-44 with the occurrence of 4.44% However, there was significance difference in the occurrence among the various age ranges at $\rho<0.05$.

Table. 1: Occurrence of Escherichia coli and Staphylococcus aureus with respect to age Range.

Age Range (Yrs)	No. of Positive Escherichia coli	Samples (%) Staphylococcus Aureus	Total Occurrence (N=90)
15-20	4(20.00)	2(10.00)	6(6.67)
21-26	4(20.00)	3(15.00)	7(7.78)
27-32	6(30.00)	4(10.00)	10(11.11)
33-38	3(15.00)	3(15.00)	6(6.67)
39-44	2(10.00)	2(10.00)	4(4.44)
Total (%)	**19(95.00)**	**14(70.00)**	**33(36.67)**

4.2 Percentage occurrence of *E. coli* and *S. Aureus* isolates from hospital subjects based on gender

Table 2 below shows the occurrence of pathogenic *E. coli* and *S. aureus* isolates based on gender. Female subject had 21.11% occurrence while male subjects had a 15.56% occurrence. This result shows statistical significant at $\rho<0.05$ in the gender.

Table. 2: Occurrence of *Escherichia coli* and *Staphylococcus aureus* with respect to gender.

GENDER	ORGANISMS E. coli	S. aureus	Total occurrence (%) [N=90]
Female	11(55.00)	8(40.00)	19(21.11)
Male	8(40.00)	6(30.00)	14(15.56)
Total (%)	**19(95.00)**	**14(70.00)**	**33(36.67)**

4.3 Marital status distribution of *E. coli* and *S. aureus* isolates from the subjects

The occurrence of pathogenic *E. coli* and *S. aureus* on the based on marital status (single or married) from the study is presented in Table 3 below. Highest positive occurrence of 21.11% was obtained from the samples collected from married outpatients while singles had an occurrence of 15.56% indicating statistically significant difference ($\rho<0.05$) between single and married samples for occurrence of *E. coli* and *S. aureus*.

Table. 3: Occurrence of *Escherichia coli* and *Staphylococcus aureus* resistance with respect to marital status.

Marital Status (%)	Organisms *Escherichia coli*	*Staphylococcus* aureus	Total (%) [N=90]
Married	11(55.00)	8(15.00)	19(21.11)
Single	8(40.00)	6(10.00)	14(15.56)
Total	**19(95.00)**	**14(70.00)**	**33(36.67)**

4.4: Antimicrobial susceptibility assay of the isolates

The result of the antimicrobial susceptibility test is presented in the table 4 below. E. coli was more sensitive to Ciprofloxacin (13.33mm) and (12.22mm) respectively and was totally resistant to amoxicilin and ampicilin while S. aureus was sensitive to amoxicilin (14.44) and ciprofloxacin and resistant to vancomycin and prefloxacin respectively. Vancomycin showed relatively low zone of inhibition on E. coli (0.44mm) whereas ampicilin demonstrated low zones of inhibition (1.11mm) on *S. aureus*.

Table. 4: Antimicrobial susceptibility assay on the isolates.

Antibiotic Agent	Diametre of Inhibition *E. coli*	*S. aureus*
Vancomycin	0.44mm	0.00mm
Amoxacilin	0.00mm	14.44mm
Ampicilin	0.00mm	1.11mm
Prefloxacin	12.22mm	0.00mm
Ciprofloxacin	13.33mm	12.22mm
Ofloxacin	15mm	13mm

DISCUSSION

Out of the ninety (90) samples analyzed, 33(36.67%) of *E. coli* and *S. aureus* were prevalent. *E. coli* had a more positive of 19(95.00%) as compared to *S. aureus* which has 14(70.00%) occurrence. The variation in the prevalence of *Escherichia coli* and *S. aureus* could be attributed to the fact that, *E. coli* is highly associated to Urinary Tract Infection (UTIs) and is the most common causes of UTIs. This was similarly reported by Tambekart *et al.* (2009), that Uropathogenic *E. coli* (UPEC) is one of the main causes of urinary tract infections (UTIs). Without argument, Adeleke & Olarinde (2013), documented that *E. coli* is the most commonly cause of UTIs in Gram-negative bacteria among all bacterial organisms associated with UTIs. Based on ages, individuals within the age groups of 27-32 years were more prevalent 10(11.11%) as compared to other age groups at $\rho<0.05$. the next age groups were 21-26 with the prevalence of 7.78% whereas the least occurred in ages groups 39-44 with occurrence of 4.44% at $\rho<0.05$. This could be due to the high sexual activity that occurred among these age groups.

On the gender basis, female subjects were higher (*E. coli* and *S. aureus*) uropathogenic organisms with percentage occurrence of 21.11% while male subjects had 15.56% indicating similarity with the report made by Jabber *et al.* (2016) which stated that women are more prone to UTIs than men possibly resulting from the fact that, females urethra is much shorter and closer to the anus than in males; they lack the bacteriostatic properties of prostatic secretions (Al-Jebouri, 1989).

The result of the antimicrobial susceptibility test is presented showed that E. coli was more sensitive to Ofloxacin Ciprofloxacin (14.44mm) (12.00mm), and prefloxacin (12.22mm) respectively and was totally resistant to amoxicilin and ampicilin while S. aureus was sensitive to amoxicilin (14.44), ofloxacion (12.22mm) and ciprofloxacin (12.22mm) while showed resistant to vancomycin and prefloxacin respectively. Vancomycin showed relatively low zone of inhibition on *E. coli* (0.44mm) whereas ampicilin demonstrated low zones of inhibition (1.11mm) on *S. aureus* which all indicates resistance following the multiple antimicrobial resistance (MAR) index.

CONCLUSION AND RECOMMENDATION

This research showed that E. coli is the most common pathogen associated with individual with urinary tract infection and is the most common causes of the infection. The infection is common among ages of 27-32 and 21-26 respectively whereas female are more prone to this infection on the gender bases.

This therefore recommends that, before an antibiotic is administered for the treatment of urinary tract infection, there is need to carry out antimicrobial sensitivity test in order to identify the most effective one drug be used.

AUTHORS' CONTRIBUTIONS

Each author contributed immensely to the writing of the manuscripts.

ETHICAL CLEARANCE

With respect to ethical clearance, all ethical issues were observed. Individual participants were informed on the purpose of their participation and concurred as their privacy was promised to be maintained.

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