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A retrospective study of antibiotic sensitivity pattern of uropathogens in the Federal Capital Territory, Nigeria

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This study was designed to bring to the fore the prevalent causative agents of urinary tract infections (UTIs) and their antibiotic susceptibility patterns in Nigeria, using patients attending University of Abuja Teaching Hospital (UATH) as a case study. Using microbiological/biochemical methods, prevalence of uropathogens amongst sexes, was compared between 2010 and 2015. Bacterial counts (10^5/ml) in the urine was used as quantitative standard and the total number of patients in the study was 214 (166 females and 48 males). The prevalence of UTIs in samples collected from 214 patients between 2010 and 2015 shows that *Escherichia coli* (57.5%) was the most prevalent organism causing UTIs, followed by *Klebsiella* species (18.5%), *Staphylococcus aureus* (11.2%) and *Proteus* species (12.4%). Antibiotics used include nitrofurantoin, ofloxacin, nalidixic acid, amoxicillin, gentamicin, tetracycline, levofloxacin, and augumentin, with bacterial causing UTI displaying greatest resistance to tetracycline and nitrofurantoin the highest sensitivity. This study indicates most of the uropathogens are still susceptible to antibiotics commonly used in the hospital. However, *E. coli* exhibited resistance to amoxicillin. The development of antimicrobials for prevention and treatment of infections should be tackled from a worldwide understanding of infection patterns so as to overcome the increasing level of superbugs in general and UTIs in particular.

Key words: Urinary tract infections, microbial resistance, adaptive immune response.

INTRODUCTION

Urinary tract infection (UTI) refers to the presence of bacteria (>10^5 bacteria per ml urine) in the urinary tract together with symptoms and sometimes signs of inflammation. UTI is one of the most commonly occurring bacterial infections among men and women (Liza and Jonathan, 2006). Due to the frequency of UTI, it necessitates more than 1.0 million hospital admissions with high economic burdens, which is estimated at $1.0 Billion U. S. Dollars (USD) of global healthcare expenditures (Foxman, 2003; Schappert and Rechtsteiner, 2007).

The prevalence of UTI varies markedly as the infection

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is problematic to all age groups. Microorganisms can reach the urinary tract by haematogenous (Daoud and Afif, 2011; Servin, 2014) or lymphatic spread (Kaper et al., 2004) via interaction between bacteria virulence and the host. Approximately 60% of all women will have a UTI during their lifetime (Daoud and Afif, 2011; Foxman, 2003).

UTIs also known as cystitis (bladder infection) when it affects the lower urinary tract and pyelonephritis (kidney infection) when it affects the upper urinary tract. In the lower urinary tract, it is characterized by burning sensation with either frequent urination or urge to urinate or both with significant pain (Nicole, 2008), although these symptoms may vary from mild to severe (Lane and Takhar, 2011; Chen et al., 2013). In healthy women, the pain lasts an average of six days (Colgan and Williams, 2011). However, in the upper urinary tract, it is characterized by flank pain, fever or nausea (Lane and Takhar, 2011; Chen et al., 2013). The most predominant etiologic agent of UTI is the *Escherichia coli* causing about 80 to 85% of the cases of UTIs, with *Staphylococcus* being the cause in 5 to 10% (Chen et al., 2013; Nicole, 2008). The prevalence of UTIs in women may be due to the proximity of the urethra to the anus (Aboderi et al., 2009; DeBacker et al., 2008). Moreover, as a woman's oestrogen hormonal level decreases due to the onset of menopause, the risk increases due to the loss of protective innate flora. In both sexes, any condition (as in the cases with diabetes, spinal cord injuries and in HIV-positive individuals) that reduces the efficacy of bladder emptying or irritates the urinary tract can cause UTIs (Samuel et al., 2012).

In Nigeria, symptomatic patients usually indulge in indiscriminate usage of antibiotics before consulting a physician. The physicians also, usually treat patients with different antibiotics without any substantive investigation (Abdorin et al., 2009). Resistance to antibiotic by bacterial and other super-bugs is an emerging and serious health problem resulting in increased morbidity and mortality (Croxen et al., 2013). In the UK alone, more than 5,500 people died from *E. coli* infections, and many of them were due to strains resistance to antibiotics (news.sky.com, 2016). The underlying molecular mechanisms for bacterial resistance to antibiotics have not been fully studied, although they are thought to include processes such as enzyme-catalyzed antibiotic modifications, bypass of antibiotic targets and active efflux of drugs from the cell (Wright, 2011; Croxen et al., 2013). Moreover, such resistance may/could also be propagated via enhanced horizontal or lateral gene transfer (LGT). LGTs can induce harmful mutations, and this can cause bacteria to resist antibiotics, creating different strains of bacteria with varying degrees of resistance due to genetic mutation (Robinson and Hotopp, 2016). UTI resistance rates against commonly prescribed antibiotics are constantly rising. For instance, it is noted that up to 20% of uropathogens are resistant to Trimethoprim/Sulfamethoxazole (TMP/SMX) and Cepholosporins. This increasing resistance is also being observed with the use of Fluoroquinolones, with resistance rates rising up to 10% (DeBacker et al., 2008).

This study was designed to evaluate UTIs and the sensitivity patterns of etiologic agents. The prevalence of UTI infections was determined using patient's bio-data (age and sex) obtained from the University of Abuja Teaching Hospital in Gwagwalada, Abuja. Also, the antibiotics susceptibility pattern to uropathogen isolates was determined.

MATERIALS AND METHODS

Aseptic collection of urine specimens

Patients collected their midstream urine in sterile bottles, closed tightly and brought to the laboratory. In the laboratory, the urine was physically analysed based on its turbidity or clearness. Bacterial counts (10^5/ml) in the urine of the patient were used as the quantitative standard of bacterial counts in the samples.

Media used

The used media included CLED agar, chocolate agar, nutrient agar for sensitivity test, peptone broth, triple sugar iron agar and Simmon's citrate agar. Each isolate was Gram-stained and subjected to biochemical tests to identify the microorganism using standard biochemical tests (Ho et al., 2004). Aseptic techniques were utilized in each of the tests.

Antibiotics

Antibiotic sensitivity test (AST) was used to determine the antibiotic that would be most successful in treating a bacterial infection using antibiotic disks (Bauer et al., 1966). The diameters of the zones of clearing around each antibiotic disk were measured in millimetres to determine the sensitivity of the isolates to the antibiotic (Bauer et al., 1966).

Quantitative analysis

Using the spread plate, urine samples were directly inoculated by streaking on the media, then incubated for 24 h at 37°C to check microbial growth.

Culture observation

Colour, size and colony morphology were observed from cultured plates. Each isolate was subjected to Gram-staining and their Gram's reaction was recorded as positive or negative.

Biochemical tests

The biochemical tests carried out for identification of the organisms were according to standard microbiological and biochemical techniques, and these tests were namely catalase, coagulase, Simmons' Citrate Test, Urea Agar Base, and Triple Sugar Iron Agar Test (Ho et al., 2004; Nwachukwu et al., 2014).

Table 1. Prevalence of uropathogens from males and females from 2010 to 2015.

Uropathogens	Prevalence (%)											
	2010		2011		2012		2013		2014		2015	
	Male	Female	Male	Female	Males	Female	Males	Female	Male	Female	Male	Female
Escherichia coli	10 (36)	18 (64)	4 (33)	8 (67)	10 (42)	14 (58)	8 (30)	19 (70)	2 (18)	9 (82)	5 (16)	27 (84)
Klebsiella spp.	2 (33)	4 (67)	2 (25)	6 (75)	1 (11)	8 (89)	0 (0)	7 (100)	0 (0)	4 (100)	1 (11)	8 (89)
S. aureus	1 (25)	3 (75)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	5 (100)	0 (0)	2 (100)	2 (18)	9 (82)
Proteus spp.	0 (0)	1 (100)	1 (33)	2 (67)	1 (33)	2 (67)	2 (22)	7 (78)	1 (50)	1 (50)	3 (27)	8 (73)

RESULTS

The prevalence of UTIs in samples collected from 214 patients between 2010 and 2015 treated at the University of Abuja Teaching Hospital of which males and females accounted for 48 (22.4%) and 166 (77.6%) of this number, respectively, are presented within this study.

Table 1 shows the total prevalence of uropathogens in both male and female patients from 2010 to June 2015 and it also indicates the mean percentage prevalence of uropathogen isolates according to gender in the years under review.

Figure 1 shows the average percentage sensitivity of the isolate to the most sensitive antibiotics tested against the various bacterial causing UTI. These antibiotics across board indicate over a 50% efficacy with *E. coli*, being the most prevalent causes of UTI, showing above 80% response as against other antibiotics, except for gentamicin, presented within this study. *Staphylococcus aureus*, however, exhibited above 80% sensitivity response to only Levofloxacin and Nalidixic acid; while, *Klebsiella* and *Proteus* species exhibited similar response to Oflaxacin and Levofloxacin. The average resistant of isolates to antibiotics as revealed from the study are as shown in Figure 2 with all the UTI isolates, which demonstrated a higher than 50% resistance. *Klebsiella* spp. exhibited a higher than 80% resistance to the antibiotics used (Figure 2), whilst *Proteus* spp. showed a 60% resistance and above, except for Cotrimoxazole; *E. coli* percent resistance was also 60% and above, except for Augmentin; while *S. aureus* was 60% and above across all the antibiotics utilized within this study.

DISCUSSION

UTIs are one of the most common infections encountered in the population with about 150 million infections estimated per year worldwide (Sharef et al., 2015). The results of the current work, indicates a number of uropathogens causing UTI including *E. coli*, *Klebsiella* spp., *Proteus*, *S. aureus* and *Candida albicans*. According to the results of this study, *E. coli* happens to be the most prevalent causative agent in all age groups and both sexes during the years are covered. *S. aureus* is a facultative anaerobic Gram-positive cocci, and it is also prevalent among patients with UTI. *S. aureus* is a commensal organism in the peri-anal and vaginal regions; therefore, emphasis on personal hygiene, most especially amongst females, may be important in reducing the UTI occurrences (O'Brien et al., 2015).

Results from this study indicates that females are the most susceptible to UTI, and this may be due to a shorter and wider urethra, which is more transversed by microorganisms, and the retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney (Kolawale et al., 2009). This result trend had also been exhibited in other studies previously carried out in Nigeria (Kolawale et al., 2009; Mbata et al., 2007).

The results indicate that ofloxacin, nitrofurantoin, nalidixic acid and levofloxacin have higher antimicrobial activity against most isolates (Figure 1) as compared to augumentin, tetracycline and amoxycilin. The high rate of bacterial resistance to the latter antibiotics may be due to the fact that they are most commonly prescribed thus their misuse may have helped conferred some form of resistance by UTI causing bacterial.

In UTI reoccurrence, the role of adaptive immune responses (AIR) has not been fully studied; hence their responses are not well understood. However, adaptive immune responses appear to contribute to immunity

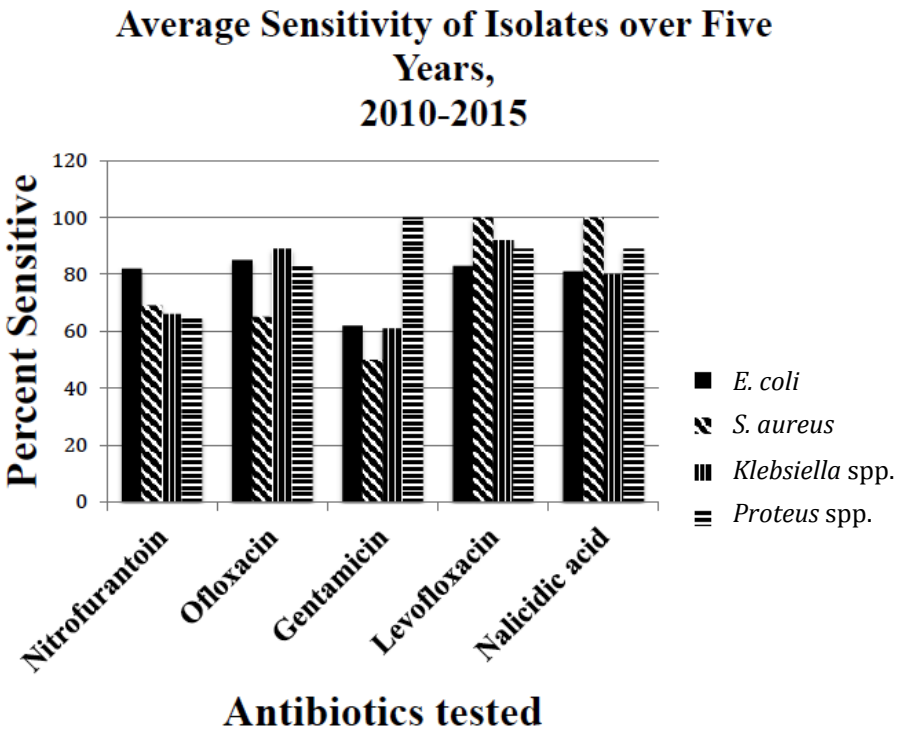

Figure 1. Average sensitivity of isolates over five years 2010-2015.

defence against UTI challenges (Thumbika et al., 2006).

This perhaps explains why some women who suffer an acute UTI do not necessarily develop a recurrent infection (O'Brien et al., 2015). Moreover, non-orthodox innate immune response to UTIs has also been established as, for instance, a novel role for yersiniabactin in UTI, which has been recently identified (Chaturvedi et al., 2013). Yersiniabactin is a siderophore, in other words a small molecule that scavenges and imports free iron, and was found to have superoxide dismutase (SOD)-like activity, hence preventing bacterial mortality in phagocytic cells that are depleted of copper and/or iron (Chaturvedi et al., 2013). To counter the effects of bacterial scavenging by transition metals, the host produces the antimicrobial protein lipocalin-2, which binds and inactivates siderophores such as yersiniabactin. It been noted that, during the onset of cystitis, lipocalin-2 protein expression is induced in the bladder epithelium (Duell et al., 2012; Taneja et al., 2008). Further, α-intercalated cells of the collecting duct present in the kidney were found to act as a molecular sieve of the upper urinary tract during cystitis, once they sensed an infection in the lower urinary tract, in a Toll-like Receptor 4 (TLR4)-dependent manner leading to the expression and secretion of lipocalin-2 into the urine filtrate (Paragas et al., 2014).

Moreover, the understanding for the role of some proteins/peptides, as defensive mechanisms against UTIs, are only becoming obvious. For instance, the protein beta defensin-1 (BD1) and Cathelicidin (LL-37) peptide shows constitutive expression in the urinary tract, as both molecules from previous studies have demonstrated to play key roles in UTI's mitigation (Mambula et al., 2000; Chromek et al., 2006). Also, in a study of uncomplicated UTI subject, elevated level of LL-37 was observed (Nielsen et al., 2014), whilst *E. coli* isolates from healthy controls exhibited more susceptibility to LL-37 than isolates from UTI patients. Further, BD1 has also exhibited constitutive expression in the urinary tract and hence, BD1 is suggested to play a role in pyelonephritis (Smith et al., 2011; Mambula et al., 2000; Morrison et al., 2002) and act as a defence against Gram-positive uropathogens (Morrison et al., 2002). Moreover, studies on defensins (Mambula et al., 2000), contained in neutrophil granules, indicated antifungal activity. These peptides combined are potential molecules of the immune system that could provide clues for the biochemical processes that can be modified to overcoming the increasing level of superbugs in general and bacterial resistance to antibiotic in UTI management in particular.

It is encouraging that the search for molecules of the immune system that could serve as alternatives to the growing resistance of some bacterial strains to antibiotics is on the increase, this is indicated in a recent study of peptides like clavin-MO (Silva et al., 2016), which have exhibited good results against strains of *E. coli* and *S. aureus*.

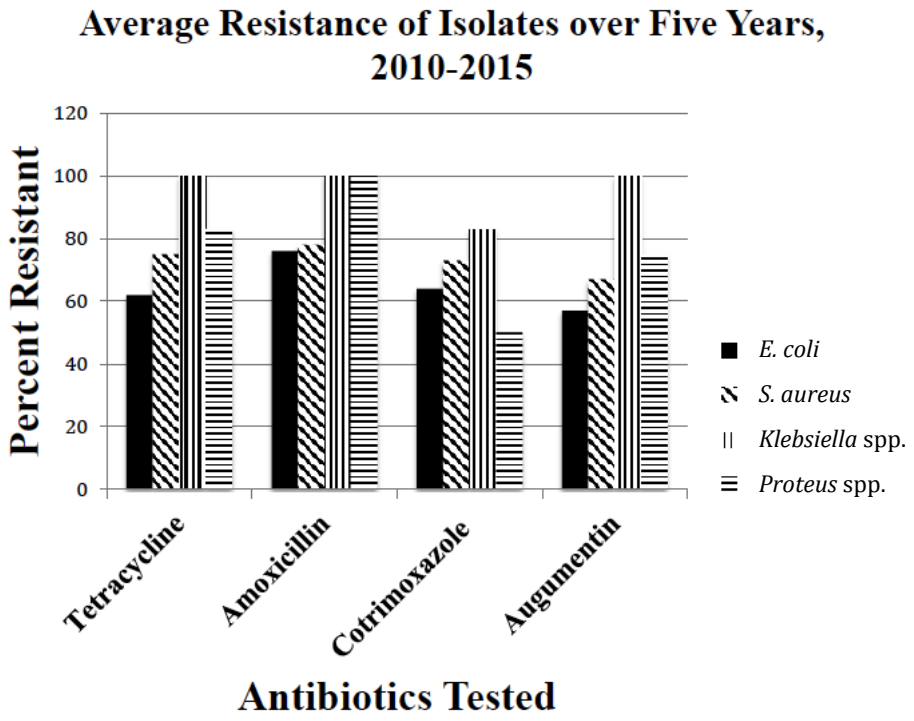

Figure 2. Average resistance of isolates over five years, 2010-2015.

From the foregone analysis, it is only reasonable that moving forward any mitigation process would require a holistic approach to include a requisite modern molecular biology tool-kit, such as Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated proteins 9 (CRISPR-Cas9), once such tool-kit has undergone the appropriate approval processes. CRISPR-Cas9 is a DNA-editing tool, which if deployed to target sequence specific moieties in the infectious bacteria, could help attenuate or inactivate production of the gene/protein conferring resistance to antibiotics. These adaptive immunity systems could in turn help to modify the host genome in the fashion of retaining the memory of past infections. Using these modern molecular tool-kits, we can only wish scientist and physicians from transition economies will be carried along, as such would enhance management of UTI and antimicrobial resistance in general.

This study indicates that most of the uropathogens causing UTIs are still susceptible to antibiotics commonly used in the hospital and community pharmacies. However, *E. coli* in particular exhibited resistance to amoxicillin and these results are similar to those of other recent studies (Kolawale et al., 2009; Tadesse et al., 2012).

Conclusions

Generally, UTI infections due to *E. coli* are thought to develop high antibiotic resistance. Thus, it is essential that effective antimicrobials for prevention and treatment of infections are developed to overcome the increasing level of superbugs in general. This is so important bearing globally; patients with infections caused by drug-resistant bacteria are at increased risk of death due to worse clinical outcome. This study was designed and aimed at bringing into focus the prevalent causative agents of UTIs and their antibiotic susceptibility patterns amongst patients attending UATH. Moving forward, it was proposed that the guideline for the approach to UTI management should include the requirement of identifying the causative organisms through urine culture and choosing the appropriate antibiotic through *in-vitro* sensitivity tests, thus down playing incessant/indiscriminate antibiotic usage. In addition, the study of antibiotic susceptibility patterns is very important for the development of empirical treatment guidelines for UTI management.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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