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ABSTRACT

The urinary tract infection is most commonly caused by gram-negative bacilli in the family Enterobacteriaceae and usually belongs to genera Escherichia, Proteus, Klebsiella, Enterobacter and Pseudomonas. The study was aimed to determine the antibiotic susceptibility profile of bacteria responsible for urinary tract infection (UTI). A total of 50 samples were collected from UTI patients attending urology clinic of Aminu Kano Teaching Hospital Kano for period of 6 month from December 2016 to May, 2017. Each of each urine sample was streaked using a sterilized platinum wire loop onto the surface of freshly prepared MacConkey and Nutrient agar plates for isolation and the isolates were identified using laboratory methods. Antibiotic susceptibility testing was conducted using Kirby-Bauer disc diffusion method. The result indicated that Escherichia coli are the most prevalent organism with total 47 occurrences accounting for 24.23%, followed by Staphylococcus aureus with total of 39 isolates (20.10), Klebsiella with 34 isolates (17.52), Pseudomonas aeruginosa with total of 29 isolates (14.95), then Proteus sp has 28 which accounted for 14.44% each while the least prevalent organisms is Staphylococcus epidermidis with 17 isolates (08.76%). Gentamicin, erythromycin and ciprofloxacin were found the most effective antibiotic for treatment of urinary tract infection. Statistical analysis of the result showed that there is considerable statistical difference in the activity of the antibiotics against the isolates at $p<0.05$. Continue surveillance of resistant rate among bacteria isolates causing UTI is needed to ensure proper recommendation for the treatment of the disease.

1. Introduction

Microorganisms are cosmopolitan and their ability to adapt to newly found environments makes them beneficial or pathogenic (Singh *et al.,* 2009). Many human diseases are as result of infections caused by pathogenic bacteria, either internal or external of the human host. One of such bacterial infection is urinary tract infection (UTI), involving the presence of bacteria in the urinary tract (UT) which is

naturally sterile (Zorc *et al*., 2005). UTI mostly occurs in patients with anatomically and functionally normal UT and usually results from spontaneous ascent of bacteria from the urethra to the bladder and occasionally, the bacteria progresses to the kidney and bloodstream (Vasudevan, 2014).

Bacteria are the prime perpetrator responsible for conferring the infection among humans but the role of certain fungi and viruses cannot be over looked. However, the incidence of UTI as a result of viral or fungal infection is considered to be rare phenomena (Demile et al., 2014). Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances. Research studies have defined urinary tract infection as the most common form of bacterial infection (Demile et al., 2014; Parveen et al., 2011).

Urinary tract infection is one of the major diseases affecting people of all age group and sex can be categorized into symptomatic and asymptomatic cases based on the pathogenesis of the infection (Azubike et al., 1994). Urinary tract infections can also be categorized as ascending and descending. Infections which are confined to the urethral or the bladder are ascending and referred to as uretitis or cystitis respectively. On the other hand, the pathogens spread from another infected body site to the kidneys down along the ureter to the bladder. Such descending urinary tract infections cause severe kidney infection, a condition known as pyelonephritis (Parsons, 1985). Bacteria are the primary organisms that cause UTI. The urinary tract infection is commonly caused by gram negative bacilli bacteria in the family Enterobacteriaceae and usually belongs to genera *Escherichia, Proteus, Klebsiella, Enterobacter* and *Pseudomonas* (Wammada et al., 2000). According to Cheesbrough (2006), Bacteria colonization of the UT is predominantly caused by Gram-negative species, such as *Escherichia coli, Klebsiella, Proteus* and *Pseudomonas* and rarely, by Gram-positive organisms such as haemolytic *Streptococci* and *Staphylococcus saprophyticus*. Gram positive bacteria cause 15-20% while negative bacteria cause 80-85% (Cheesbrough, 2006). Among gram negative *Escherichia coli* is the most frequent pathogen (Gales *et al*., 2002) but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Seratia, Enterobacter* and *Pseudomonas*. Among gram positives *S. saprophyticus, E. faecalis, S. pyrogenes, and S. aureus* are usually prevalent and are resistant to variety of antibiotics (Thomas, 1995). *Enterococcus* isolates cause 2.3% of UTI and best known as antibiotic resistant Opportunistic pathogen (Rizk et al., 2001).

UTI is the most frequent nosocomial infection and has been suffering a shift in the etiology and antimicrobial susceptibility, as common as other infection in the last decade. It is important to know the etiology and antibiotic susceptibility of infectious agents to guide the initial empirical treatment (Roberts and Akintemi, 1999). Prevalence of urinary pathogens and their susceptibility to commonly used antibiotics varies regionally, therefore it become necessary to have knowledge of prevalence of these organisms and their susceptibility to antibiotics in a particular setting (Mussa-Aisien *et. al*., 2003). The study was aimed to determine the antibiotic susceptibility profile of bacteria isolated from UTI patients in Kano, Nigeria.

2. Materials and methods

Study Area

The study was conducted at Urology clinic of Aminu Kano Teaching Hospital Kano (AKTH). Kano state is located in the North-west Nigeria with coordinates 11^0 30 N 8^0 30 E. It shares borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the West and Niger republic to the North. It has a total area of 20,131km^2 (7,777sqm) and population of 11,058,300 (NPC, 2006).

Ethical clearance

An approval for the study was obtained from Research and Ethic committee of Aminu Kano Teaching Hospital Kano. The aim of the study was explained clearly to the clients and informed consent obtained before proceeding to the study.

Study Population

A total of 50 samples were collected from UTI patients attending urology clinic of Aminu Kano Teaching Hospital Kano for period of 6 month from December 2016 to May, 2017. The inclusion criteria for the study include male adult with Urinary Tract Infections UTIs.

Samples Collection

Early morning mid-stream urine samples of about ten (10) ml were collected using clean and sterilized plastic bottles with air-tight screw cap tops. Each urine sample bottle was labelled with a reference code, age, sex, and time of collection. The samples were placed in a cold box for transportation to the laboratory, where it was stored until analyses were carried out. All samples were analyzed with the microbial culture method and conventional urine analysis.

Culturing, Isolation and identification of Bacteria

Each of each urine sample was streaked using a sterilized platinum wire loop onto the surface of freshly prepared MacConkey and Nutrient agar plates. The plates were incubated at 37^0C for 24 hours to isolate the growing microorganisms. Representative of growing colonies were picked with a sterile wire loop and re-inoculated onto the surface of nutrient agar, pure cultures were made with repeated streaking. The resulting pure colonies obtained were used for biochemical tests aimed at identifying the bacteria isolates. Isolates were particularly subjected to Gram staining, indole, citrate utilization, catalase, urease, methyl-red, Voges Proskauer and coagulase test (Holt et al., 1994)

Antibiotic Susceptibility Testing

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method as described by Bauer *et al*. (1996). Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to Ampicillin (30 µg), Streptomycin (30 µg), Augmentin (10 µg), Erythromycin (30 µg), Amoxacillin (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tetracycline (30 µg), Ciprofloxacin (10 µg) and Septrin (30 µg) produced by Abtek pharmaceutical limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediate susceptible and resistant according to the European committee on antimicrobial susceptibility testing (EUCAST) breakpoint for interpretation of MICs and zone diameters (2015).

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using One-Way ANOVA and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). Significance level for the differences was set at $p<0.0$

3. Results

Demographic distribution of the subjects

The Demographic distribution of the subjects is presented in Table 1. A total of 50 subjects participated in the study, all of which are patients diagnosed with urinary tract infection. The age category, sex, marital status and type of resident were considered for demographic distribution of the subjects.

Table 1: Demographic distribution of the subjects with percentage prevalence

Parameters	Number	Prevalence (%)
Age (years)		
Less than 18	01	02
18 – 40	16	32
41 – 70	28	56
70 – above	05	10
Sex		
Male	21	42
Female	29	58
Marital status		
Single	08	16
Married	42	84
Types of resident		
Urban	09	18
Semi-urban	17	34
Rural	24	48

Prevalence of Bacterial Isolates

The prevalence of bacteria isolated from the urine samples of UTI patients attending urology clinic of Aminu Kano Teaching Hospital is presented in Table 2. The result indicated that *Escherichia coli* are the most prevalent organism with total 47 occurrences accounting for 24.23%, followed by *Staphylococcus aureus* with total of 39 isolates (20.10), *Klebsiella* with 34 isolates (17.52), *Pseudomonas aeruginosa* with total of 29 isolates (14.95), then *Proteus* sp has 28 which accounted for 14.44% each while the least prevalent organisms is *Staphylococcus epidermidis* with 17 isolates (08.76%).

Table 4.2: Prevalence of isolated from urine samples of UTI patients

Organisms	No. of occurrence	Percentage occurrence (%)
E. coli	47	24.23
S. aureus	39	20.10
Klebsiella species	34	17.52
P. aeruginosa	29	14.95
Proteus species	28	14.44
S. epidermidis	17	08.76

Antibiotic Sensitivity Testing

The result of antibiotic sensitivity testing of the isolates against some antibiotics is presented in Table 3. The antibiotics used include Ampicillin, Streptomycin, Augmentin, Erythromycin, Amoxacillin, Chloramphenicol, Gentamicin, Tetracycline, Ciprofloxacin and Septrin. The result showed that most of the isolates were sensitive to the antibiotics used.

Susceptibility and Resistivity Status of the Isolates

The susceptibility and resistivity status of the isolates is presented in table 4. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediate susceptible and resistant according to the European committee on antimicrobial susceptibility testing (EUCAST) breakpoint for interpretation of MICs and zone diameters (2015).

Table 3: Antibiotic Susceptibility Profile of the Isolates against the Antibiotics Used

		Bacteria/zone of inhibition (mm)					
Antibiotics	**Conc.**	***E. coli***	***Klebsiella***	***Proteus***	***Pseudomo***	***S. aureus***	***S. epidim***
Ampicillin	30 µg	16 ± 0.8^{b}	10 ± 0.0^{a}	10 ± 0.0^{a}	10 ± 0.0^{a}	21 ± 1.6^{c}	19 ± 1.1^{c}
Streptomycin	30 µg	21 ± 1.5^{b}	19 ± 1.2^{b}	21 ± 1.7^{b}	20 ± 1.9^{b}	10 ± 0.0^{a}	10 ± 0.0^{a}
Augmentin	10 µg	21 ± 0.9^{b}	23 ± 1.8^{b}	21 ± 1.1^{b}	10 ± 0.0^{a}	21 ± 1.3^{b}	20 ± 0.7^{b}
Erythromycin	30 µg	19 ± 1.2^{a}	22 ± 1.9^{ab}	20 ± 1.6^{a}	21 ± 0.8^{a}	23 ± 0.9^{b}	21 ± 1.0^{a}
Amoxicillin	30 µg	23 ± 1.1^{c}	22 ± 1.5^{a}	18 ± 1.6^{b}	10 ± 0.0^{a}	22 ± 1.0^{c}	19 ± 1.3^{b}
Chloramph.	30 µg	21 ± 0.7^{b}	10 ± 0.0^{a}	21 ± 1.2^{b}	10 ± 0.0^{a}	23 ± 1.5^{b}	21 ± 1.3^{b}
Gentamicin	10 µg	20 ± 1.3^{a}	23 ± 1.0^{b}	22 ± 1.4^{ab}	21 ± 1.9^{a}	20 ± 1.0^{a}	21 ± 0.6^{a}
Tetracycline	30 µg	23 ± 1.0^{b}	21 ± 0.9^{b}	10 ± 0.0^{a}	10 ± 0.0^{a}	10 ± 0.0^{a}	10 ± 0.0^{a}
Ciprofloxacin	10 µg	24 ± 1.9^{c}	20 ± 1.4^{b}	22 ± 1.1^{b}	23 ± 1.2^{bc}	19 ± 0.7^{a}	16 ± 0.4^{a}
Septrin	30 µg	22 ± 1.8^{b}	21 ± 1.0^{b}	23 ± 1.4^{c}	10 ± 0.0^{a}	22 ± 1.8^{b}	20 ± 1.1^{b}

Key: values having different superscript on the same row are considered significantly different at $p<0.05$

Table 4: Antibiotic Susceptibility Profile of the Isolates against the Antibiotics Used

Antibiotics	**Conc.**	***E. coli***	***Klebsiella***	***Proteus***	***Pseudomo***	***S. aureus***	***S. epidim***
Ampicillin	30 µg	I	R	R	R	S	S
Streptomycin	30 µg	S	S	S	S	R	R
Augmentin	10 µg	S	S	S	R	S	S
Erythromycin	30 µg	S	S	S	S	S	S
Amoxicillin	30 µg	S	S	I	R	S	S
Chloramph.	30 µg	S	R	S	R	S	S
Gentamicin	10 µg	S	S	S	S	S	S
Tetracycline	30 µg	S	S	R	R	R	R
Ciprofloxacin	10 µg	S	S	S	S	S	I
Septrin	30 µg	S	S	S	R	S	S

Key: S = Sensitive, I = intermediate sensitive and R = resistance.

4. Discussion

The study was aimed to isolate bacteria responsible for urinary tract infection (UTI) and to determine antibiotic susceptibility profile of the isolates. A total fifty (50) urine samples collected from UTI patients were analyzed using standard method to isolate and characterized bacteria causing UTI. The study revealed that urinary tract infection is polymicrobial infection caused by both Gram positive and gram negative bacteria. Six bacteria were isolated in the present study namely; *Escherichia coli, Staphylococcus aureus, Klebsiella, Pseudomonas aeruginosa, Proteus* sp and *Staphylococcus epidermidis.* In this study, *Escherichia coli* is the most prevalent specie which accounted for 24%, this is followed by *Staphylococcus aureus* with 20% prevalence, then *Klebsiella* with prevalence of 17%, *Pseudomonas aeruginosa* 15% and *Proteus* sp 14% while least prevalent isolate is *Staphylococcus epidermidis* accounted for 8%. Several studies were conducted to identify bacteria isolates responsible for urinary tract infection which correlate and agree with the present study. In a study conducted by Kodna and Gupta, (2010) *E. coli* is the predominant uropathogen isolated in acute, community-acquired uncomplicated UTIs in adults and children. Other uropathogens include *Staphylococcus* sp, and the *Enterococcus, Klebsiella, Enterobacter* and *Proteus* genus. *Escherichia*

coli and the *Proteus*, *Klebsiella*, *Pseudomonas*, *Serratia* and *Enterococci* genus are the usual strains found. This finding was inconformity with the present study. The treatment strategy for complicated UTIs depends on the severity of the illness and hospitalization is often necessary (Grabe et al., 2016).

According to Chakupurakal et al. (2010), the predominant pathogen responsible for UTI is *E. coli* which constitutes up to 80-85% and is followed by *Staphylococcus* which accounts to 5-10%, this is in line with the present study. The occurrence of the infection due to viral or fungal agents is a rare phenomenon. In addition to the above mentioned bacterial species, *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter* are associated with UTI. The bacteria enter the bladder through urethra and the infection can also occur through blood and lymph. This also supported the finding of the present study. The Gram-negative rods *Escherichia coli* is commonest cause of ascending UTIs about 60-90%; this is probably because they are often present in the colon and virulence factors which include: the possession of K antigens and specialized fimbriae (Cheesbrough, 2009).

The antibiotic susceptibility profile of the isolate in this study indicated that most of the isolates were susceptible to the antibiotics used. According to this study, *Escherichia coli* is the most susceptible organism with average zone of inhibition of 21 mm, this is followed by *S. aureus* with 19.1 mm, *Proteus* 18.8 mm, *Klebsiella* 18.1 mm while the least sensitive organisms are *S. epidermidis* 12.6 mm and *Pseudomonas aeruginosa* 13.5 mm. The finding of this study showed that Gentamicin is the most effective antibiotic for treatment of urinary tract infection. In addition to that, erythromycin and ciprofloxacin were also highly active against the isolates, followed by septrin, augmentin and amoxicillin. This finding was inconformity with the finding of Theodore 2007 who found ciprofloxacin, chloramphenicol and erythromycin. Statistical analysis of the result showed that there is considerable statistical difference in the activity of the antibiotics against the isolates at $p<0.05$

5. Conclusion

The study revealed that urinary tract infection is polymicrobial infection caused by both Gram positive and gram negative bacteria. The *Escherichia coli* are the most prevalent organism with total 47 occurrences accounting for 24.23%, followed by *Staphylococcus aureus* with total of 39 isolates (20.10), *Klebsiella* with 34 isolates (17.52), *Pseudomonas aeruginosa* with total of 29 isolates (14.95), then *Proteus* sp has 28 which accounted for 14.44% each while the least prevalent organisms is *Staphylococcus epidermidis* with 17 isolates (08.76%). Gentamicin, erythromycin and ciprofloxacin were found the most effective antibiotic for treatment of urinary tract infection. It is recommended that continue surveillance of resistant rate among bacteria isolates causing UTI is needed to ensure proper recommendation for the treatment of the disease.

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