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Research Article

Determination of Antibiotic Sensitivity Pattern of Bacteria Associated with Urinary Tract Infection (UTI) Among Adult Males in Kano, Nigeria

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Abstract: Urinary tract infections (UTIs) is one of the serious infection that affects human population especially men. The study was aimed to characterize and determine the antibiotic sensitivity pattern of bacteria associated with urinary tract infection among adult patients in Kano, Nigeria. A total of 200 samples were collected from adult male patients attending urology clinic of Aminu Kano Teaching Hospital Kano for period of 6 month from December 2016 to May, 2017. The samples were inoculated on plates of Cystine Lactose Electrolyte Deficient media (CLED) by method of streaking. Cultures were incubated at 37°C aerobically overnight for bacterial isolation. Isolates were identified using Gram staining, biochemical (catalase, coagulase, DNase, indole, methyl-red, VP, Citrate utilization and urease) tests and motility test. The bacteria isolates were subjected to antibiotic susceptibility testing using agar disc diffusion method. The result indicated that *E. coli* is the most prevalent isolate with total 105 occurrences accounting for 35%. This is followed by *Klebsiella* 66 (22%), *P. aeruginosa* 27 (9%), *Staphylococcus aureus* 26 (8.66%), *Proteus* sp 23 (7.66%), *E. faecalis* 22 (7.34%), *S. epidermidis* 20 (6.67%) while the least prevalent organism is *Salmonella* sp 11 (3.67%). The highest susceptibility was recorded in Perfloxacin antibiotic with highest susceptibility to *E. coli*, *Proteus*, *P. aeruginosa and Staphylococcus aureus* to highest resistance was in Chloramphenicol with about 90% resistance to the bacterial isolates. It is concluded that bacteria is one of the major causative agent of urinary tract infections.

Keywords: Antibiotics, bacteria, sensitivity, urinary tract infections.

INTRODUCTION

Microorganisms form a bulk of the earth's biomass and their ability to adapt to newly found environment makes them beneficial or pathogenic (Singh *et al.*, 2011). Many human diseases are as a result of infections caused by bacteria pathogens, either external or internal of the human host. One of such bacterial infection is the 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. As the name indicates, the infected parts involve the urinary tract comprising of the upper and lower urinary tract. The infection is named after the part that gets infected and is referred to as cystitis (bladder infection) and pyelonephritis (kidney infection) (Vasudevan, 2014). The symptoms associated with the bladder and kidney infections are contrasting which includes painful and frequent urination in case of cystitis as a result of bladder infection whereas conditions like high fever and flank pain are commonly experienced in case of pyelonephritis (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. 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 (Demile *et al*., 2014). Research studies have defined urinary tract infection as the most

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common form of bacterial infection (Parveen *et al*., 2011).

Urinary tract infection can be a consequence of poor diagnosis and is regarded as the common hospital acquired infection (Koffuor *et al*., 2012; Kolawale *et al*., 2009). The infection encompasses a diverse group of clinical syndromes and diseases that differ in epidemiology, etiology, location severity of the condition (Lucas and Cunningham, 1993). Urinary tract infection is one of the major diseases that affect people of all age groups and sexes and can be separated into asymptomatic and symptomatic cases based on the pathogenesis of infection (Azubike *et al*., 1994). Proliferation of bacteria in the urinary tract is the cause of urinary tract infection. 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* (Wammada *et al*., 2000). 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* (Cheesbrough, 2010).

Gram positive bacteria cause 15-20% and gram negative bacteria cause 80-85%. 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, Serratia, 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 (Murray, 2000). UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC are followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophytic*, *Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida spp., S. aureus, P. mirabilis* and *P. aeruginosa* (Ali *et al*., 2018). The study was aimed to characterize and determine the antibiotic sensitivity pattern of bacteria associated with urinary tract infection among adult patients in Kano, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted at Urology clinic of Aminu Kano Teaching Hospital Kano (AKTH). Kano lies between Latitude 11.9^0 North and Longitude 8.5^0 East in North western Nigeria. It is bounded to the north by Katsina State, to the east and south by Jigawa State and to the west by Kaduna state. Kano State 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 300 samples were collected from adult male 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.

Determination of Sample Size

Sample size for the study was determined from a standard formula for the calculation of minimum sample size (Waiya et al., 2018). Sample size was given by the formula:

$N = \frac{Z^2pq}{D^2}$

N = Desired sample size

S = Standard deviation at 95%

Z = 1.96 Confidence interval

D = 0.05, mean deviation n at 95%

P = estimated/expected prevalence from previous study in area

P = previous prevalence=58%=0.58

From the above formula $N = \frac{Z^2pq}{D^2}$

P = 0.58

Q = 1 – P = 1- 0.58 = 0.32

$N = \frac{(1.96)^2\ (0.58 \times 0.52)}{(0.5)^2}$

$N = \frac{3.843 \times 0.185}{0.0025}$

$N = \frac{0.7109}{0.0025} = 284$

Samples Collection

Early morning mid-stream urine samples of about 10 ml were collected using clean and sterilized plastic bottles with air-tight screw cap tops. Each urine sample bottle was labeled 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 and Identification of Isolates

The samples were inoculated on plates of Cystine Lactose Electrolyte Deficient media (CLED), by method of streaking. Cultures were incubated at 35-37°C aerobically for overnight. Isolates were identified using Gram staining, biochemical (catalase, coagulase, DNase, indole, methyl-red, VP, Citrate utilization and urease) tests and motility test as described by Cheesbrough (2010).

Sensitivity Testing

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc 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 Sparfloxacin (30 µg), Streptomycin (30 µg), Augmentin (10 µg), Perfloxacin (30 µg), Amoxacillin (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tavavid (30 µg), Ciprofloxacin (10 µg) and Septrin (30 µg), produced by Abtek pharmaceutical limited, were determined. The plates were examined for zones of inhibitions and the values were recorded in millimeters.

RESULTS

Identification of Bacteria Isolates

The Isolates were identified based on their colony morphology, shape and Biochemical reaction. For suspected *E. coli* colony, Under Light Microscope the smear appeared to be pink colored Rod-like structure which shows Organism is Gram negative, for suspected *Klebsiella sp.* smear appears to be short pink colored Rods which shows organism is gram negative, *Proteus sp* appeared as pink colored rods, which shows organism is Gram negative, *Staphylococcus aureus* appeared as purple cocci in clusters with grape like appearance signifying the Organism is gram positive cocci, *Staphylococci epidermidis* appeared as Purple cocci, which signify it is Gram Positive, *Pseudomonas aeruginosa* and *Salmonella sp* when viewed appeared as pink short rods in singles, signifying it is gram negative and *Enterococci faecalis* when the smear was viewed under light Microscope appeared as Purple cocci signifying it is gram positive.

Table 1: Morphological and Biochemical characterization of Bacteria Isolates

Colonial morphology	G/S	CAT	IND	MOT	CIT	MR/VP	Suspected Organism
Opaque yellow milky growth	+	+	-	-	-	+/-	*Staphylococcus aureus*
Flat circular pink colonies	+	+	-	-	-	+/-	*Staphylococcus epidermises*
Dark centered with green metallic sheen colonies	-	+	+	+	-	+/-	*Escherichia coli*
Abundant, thin, white growth with medium turning green	-	+	-	-	-	-/-	*P. aeruginosa*
Circular black centered colonies	-	+	-	+	+	-/+	*Salmonella Sp*
Translucent creamy mucoid round	-	-	-	-	+	-/+	*Klebsiella Sp*
Thin, blue/gray, spreading growth	-	+	+	+	+	+/-	*Proteus Sp*
Clear, smooth small, round colony	-	-	-	-	-	+/-	*Enterococcus faecalis*

Key: G/S = Gram staining, CAT = Catalase, IND = Indole, MOT = Motility, CIT = Citrate, MR = Methyl red, VP = Voges proskauer .

Prevalence of Bacterial Isolates

The prevalence of bacteria isolated from the urine samples of UTI male adult patients attending Urology clinic of Aminu Kano Teaching Hospital is presented in Table 2. The result indicated that following biochemical identification of the isolates, amongst which Gram negative Bacteria were the predominant organisms isolated from the urine sample collected i.e. 254 (84.6%) while Gram positive bacteria are 46 (15.4%). *E. coli* is the most prevalent isolate with total 105 occurrences accounting for 35%. This is followed by *Klebsiella* 66 (22%), *P. aeruginosa* 27 (9%), *Staphylococcus aureus* 26 (8.66%), *Proteus* sp 23 (7.66%), *E. faecalis* 22 (7.34%), *S. epidermidis* 20 (6.67%) while the least prevalent organism is *Salmonella* sp 11 (3.67%).

Table 2: Prevalence of bacteria isolated from urine samples of UTI patients

Organisms	No. of occurrence	Percentage occurrence (%)
E. coli	105	35.00
Klebsiella species	66	22.00
Proteus species	23	07.66
S. epidermidis	20	06.67
E. faecalis	22	07.34
P. aeruginosa	27	09.00
S. aureus	26	08.66
Salmonella species	11	03.67
Total	**300**	**100**

Sensitivity Pattern of the Isolates

Table 3 shows the susceptibility pattern of the distribution of the isolates across various antibiotics used in AKTH. The highest susceptibility was recorded in Perfloxacin antibiotic with highest susceptibility to *E. coli, Proteus, P. aeruginosa and Staphylococcus aureus* to highest resistance was in Chloramphenicol with about 90% resistance to the bacterial isolates. *E. coli* highest susceptibility to Perfloxacin and Chloramphenicol, *Klebsiella* species, highest susceptibility was recorded in Streptomycin and resistance to Ofloxacin, *Proteus* was susceptible to Amoxicillin and Resistance to chloramphenicol, recorded 100% resistance highest resistance was in Gentamicin with about 90% resistance to the bacterial isolates. *E. coli* was resistant to three antibiotics (Augmentin, Amoxicillin, and Chloramphenicol). Ciprofloxacin was sensitive to gram negative Bacterial isolates but Resistant to *Klebsiella and E. faecalis.* Most of the isolates where resistant to, Gentamicin and septrin.

Table 3: Sensitivity Pattern of the Isolates against some Antibiotics

	Antibiotics/Number and percentage of sensitive isolates							
biotics	***E. coli***	***Klebsiella***	***Proteus sp***	***P. aeruginosa***	***E. faecalis***	***Salmonella***	***S. aureus***	***S. epidermidis***
SPA	73(69)	0(0)	24(100)	19(83)	27(100)	0(0)	3(16)	4(17)
STR	39(37)	0(0)	21(87)	27(100)	4(14)	0(0)	3(16)	12(50)
AUG	14(13)	24(100)	0(0)	0(0)	27(100)	0(0)	18(100)	0(0)
PER	24(100)	15(25)	24(100)	27(100)	0(0)	0(0)	18(100)	0(0)
AMO	28(27)	0(0)	24(100)	27(100)	0(0)	0(0)	18(100)	0(0)
CHL	0(0)	0(0)	0(0)	27(100)	0(0)	0(0)	9(50)	12(50)
GEN	24(23)	6(25)	24(100)	0(0)	6(28.5)	0(0)	0(0)	12(50)
OFL	19(18)	0(0)	24(100)	0(0)	0(0)	1(12.5)	10(77)	12(50)
CIP	43(41)	0(0)	24(100)	0(0)	0(0)	12(100)	8(33)	16(67)
SEP	28(28)	24(100)	24(100)	0(0)	0(0)	0(0)	(0)	0(0)

Key: SPA = Sparfloxacin, STR = Streptomycin, AUG = Augmentin, PER = Perfloxacin, AMO = Amoxicillin, CHL = Chloramphenicol, GEN = Gentamicin, OFL = Ofloxacin, CIP = Ciprofloxacin, SEP = Septrin.

DISCUSSION

The result of Isolation and identification of the isolated revealed 8 bacterial Species. *E. coli* was most prevalence with 105 (35%), *Klebsiella* 66 (22%), *Proteus species* 24 (8%), S*taphylococcus epidermidis* 24(8%), *E. faecalis* 24 (8%), *Pseudomonas aeruginosa* 27(9%), *Staphylococcus aureus* 18(6%), and lowest prevalence was in *Salmonella* 12 (4%). The result of this study was inconformity with that of Lavison and Kaye (2013) who found that for the agents involved in uncomplicated UTIs include *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida spp., S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS (Bagga *et al.*, 2003). The prevalence of *E. coli* (32.5%) in the present study is comparable with that reported with that of Nas *et al*. (2018), who found the high prevalence of *E. coli* among UTI patients in Kano, Nigeria. On the other hand, the result of this study was in contrary to that of the result reported by Girun (2012) from Diredawadilchora Hospital and that of Fantahun and Bayeh (2009) at Felege Hiwot referral Hospital who found the percentage prevalence of *E. coli* of 53% and 48% respectively. The result of the present study showed that more than one bacterial species (mixed type) were isolated which reported that mixed infection (poly-microbial) are more likely to occur in patients with underlying disorders that interfere with free urine flow and are frequent in those with indwelling catheter. The similarities and difference the type and distribution of uropathogens may result from different environmental conditions and the prevailing practice in each country and region. This difference in prevalence could be based on differences in sanitary conditions and observed personal hygiene. Again, the higher prevalence of *E. coli*-causing UTI in adults than the observed value may be due to sexual activity (Geerlings *et al.*, 2000).

The results also revealed that among ten antibiotics used for susceptibility test Perfloxacin was the most effective antibiotics with over 90% and highest resistance was in Gentamicin .This is in contrast with a finding by (Ronald and Harding, 2007) that says that Bacteria known as *E. coli* cause the majority of lower urinary tract infections. This microorganism is usually susceptible to a variety of antibiotics, such as Trimethoprim and ciprofloxacin. In the present study, multiple antibiotics resistance were also shown on many of the identified species, thus, *E. coli* were resistant to more than three antibiotics (Augmentin, Amoxicillin and Chloramphenicol) while the rest of the isolates were resistant to three to four antibiotics. Ciprofloxacin was highly active against gram negative bacteria isolates but resistant to *Klebsiella* and *E. faecalis*, this agrees with (Theodore, 2007). Perfloxacin also showed high sensitivity to the isolated but resisted by *Klebsiella*. Most of the isolates were resistant, Gentamycin and Septrin. The higher resistance against the above antimicrobials could be as a result of repeated or prolonged use or exposure of uropathogens to antibiotics (Hiller, 2007; Sing, 2006). Repeated use

of antibiotics can damage urethral flora, allowing uropathogens to colonize and subsequently to Re-infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI. Moreover, this condition enables bacteria to exchange their genetic material through horizontal gene transfer resulting in resistant gene that confer resistance to a particular antibiotic (Tessema and Tanagho, 2007).

CONCLUSION

Based on the findings of the study, the following bacteria were isolated from UTI male adult patients; *Escherichia coli*, *Klebsiella sp*, *Proteus sp*, *Pseudomonas aeruginosa*, *Salmonella sp*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* with *E. coli* being most prevalent isolate with total 105 occurrences accounting for 35%. This is followed by *Klebsiella* 66 (22%), *P. aeruginosa* 27 (9%), *Staphylococcus aureus* 26 (8.66%), *Proteus* sp 23 (7.66%), *E. faecalis* 22 (7.34%), *S. epidermidis* 20 (6.67%) while the least prevalent organism is *Salmonella* sp 11 (3.67%). . Most of the isolates were sensitive to Perfloxacin, Ciprofloxacin, Sparfloxacin, and resistant to Gentamicin and Septrin.

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