



MULTIDRUG RESISTANCE PATTERN OF URINARY BACTERIA ISOLATED FROM URINE OF PATIENTS ATTENDING SPECIALIST HOSPITAL BAUCHI

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ABSTRACT

In this study, clean-catch midstream urine of patients with clinical symptoms were sampled from patients attending Specialist Hospital Bauchi. Urine specimens were cultured for isolation of the microbial agents of urinary tract infection (UTI). The isolated bacteria were identified using biochemical test and Disk diffusion susceptibility test were used to determine the susceptibility of bacterial agents to antibiotics. Out of the 123 samples collected, 37 yielded no growth while 86 yielded growth. The total bacteria isolated were 91 isolates because some samples yielded multiple bacteria. *Staphylococcus aureus* 26 (28.6%) were the commonest pathogen isolated followed by *Klebsiella spp* 25 (27.5%), *Escherichia coli* 18 (19.8%), diptheriod 7 (7.7%), *Candida spp* 6 (6.6%) followed by coagulase negative *Staphylococcus* 3 (3.3%), *Streptococcus spp* 3 (3.3%), *Proteus spp* 2 (2.2%) and *Pseudomonas* 1 (1.1%) as the least. *Staphylococcus aureus* showed high sensitivity to Chloramphenicol (69.2%) and *Klebsiella spp* revealed high sensitivity to streptomycin (24.0%). *Staphylococcus aureus* was most resistant to cloxacillin, ampiclox and amoxicillin while *Klebsiella spp* was most resistant to augumentin. UTI was more prevalent among female (70.6%) than male (29.6%). This study justifies the resistance pattern of some microorganisms isolated from patients with UTIs.

Key words: multidrug resistance, Urinary Tract Infection, bacteria and Disk diffusion

1.0 INTRODUCTION

Urinary tract infection (UTI) occurs when bacteria enters the urinary tracts through the urethra and begins to multiply in the bladder (Mayo, 2016). Although, the urinary system is designed to keep out such microscopic invaders, these defenses sometimes fail. When this happens, bacteria may take hold and grow in full-blown infection in the urinary tract (Mayo, 2016).

Urinary tract infection is the most common bacterial infection in humans (Nicolle, 2002). It is also the most common infectious presentation in community medical practice (Amin *et al.*, 2009) and also the leading cause of gram negative species in hospitalized patients (Annabelle *et al.*,). Urinary tract infections comprises of both asymptomatic microbial colonization of the urine and symptomatic infection with microbial invasion and inflammation of urinary tract structures (Ochada *et al.*, 2014).

The most common cause of urinary tract infection is *Escherichia coli* though other bacteria such as *Klebsiella* spp., *Staphylococci* spp., *Enterococci* spp., *Pseudomonas* spp., *Proteus* spp. and *Enterobacter* spp. can also cause urinary tract infection (UTI) (Jelheden *et al.*, 1991, Ronald, 2002 and Bronsema *et al.*, 1993). Risks factors of UTI includes; sexual intercourse, diabetes, obesity and family history (Flores-Mireles, 2015). Although, sexual intercourse is a risk factor UTI are not classified as sexually transmitted infections (EHS, 2013).

UTIs are classified into complicate and uncomplicated UTIS (Stamnet *al.*, 2012). Uncomplicated UTIs occurs in sexually active healthy female patient with structurally and functionally normal urinary tracts (Amin *et al.*, 2009), while complicated UTIs are associated with co-morbid conditions that prolongs the need for treatment or increases the chances for therapeutic failure. (Amin *et al.*, 2009). These conditions include abnormalities of the urinary tract that impede urine flow, the existence of a foreign body or infection with multi drug resistance of pathogens (Amin *et al.*, 2009). UTIs in male patients are considered complicated. Despite the involvement of the upper urinary tract, pyelonephritis can be considered uncomplicated when it occurs in healthy patient (Hooton, 2000 and Stapleton, 2003).

Multidrug resistance organisms are defined as microorganisms predominantly bacteria that are resistant to one or more classes of antimicrobial agents, antimicrobial resistance is a global concern because new resistant mechanism emerge and spread globally threatening the ability to treat common infectious disease which result to death and disability of individuals who could continue a normal course of life (WHO, 2015).

The etiology of UTI and the antimicrobial susceptibility of urinary pathogens in both communities and hospitals have been changing and in recent years, antibiotic resistance has become a major problem worldwide due to several factors related to the genetic nature of the organisms and selective antimicrobial pressure in humans (Ochada *et al.*, 2014). Currently, the prevalent pathogen of UTI has been resistant to most chemotherapeutic agents making antimicrobial susceptibility highly unpredicted without laboratory procedures. These would have profound impact on future management of infection with these drugs. Hence, it is necessary to ascertain the current multidrug resistance trends of UTIs so as to provide empherical

information that will ease diagnosis and established suitable antibiotics for use. The aim of the study is to assess the multidrug resistance pattern of bacteria isolated from urine of patients attending Specialist Hospital Bauchi.

MATERIALS AND METHOD

StudyArea

This study was carried out in Bauchi state, North eastern Nigeria. The samples were collected from Specialist Hospital Bauchi and transported to the microbiology laboratory of Science Laboratory Technology department, Federal Polytechnic Bauchi for analysis.

Sample Collection

The first urine passed by the patients at the beginning of the day was collection in a sterile bottle and sent for examination. The specimen is the most concentrated and therefore the most suitable for culture macroscopy, microscopy and biochemical analysis. Midstream urine for microbial analysis were collected in sterile, dry, wide necked plastic bottles. 123 sterile urine samples were collected for bacteriological analysis.

Preparation and Identification of Wet Mount

Ten milliliters of urine was aseptically transferred into a labeled conical tube. It was centrifuged at 500-1000g for 5 minutes and the supernatant fluid was turned into a second container (for identification). The preparation was examined microscopically using 10X and 40X objective (Cheesbrough, 2006).

Culture and sensitivity

A sterile wire loop was used for inoculating into prepared culture plates. A loop full of urine was streaked on a dried CLED agar media, MacConkey and blood agar. The plate was incubated aerobically at 37⁰C for 24 hours after which biological tests were done and were then sub-cultured into nutrient agar media to carry out sensitivity for another 24 hours at 37⁰C (Cheesbrough, 2006).

Characterization of Isolates

The various isolates identified were subjected to morphological and biochemical tests were carried out according to standard methods. The various biochemical tests were carried out to identify the isolates obtained include catalase, coagulase, indole, citrate, methyl red. ((Cheesbrough, 2006; Ochei *et al.*, 2000).

Antibiotic Susceptibility test

Antibiotic susceptibility of pure culture of confirmed isolate were performed on nutrient agar by the Kirby bear disc diffusion method, using gram positive and gram negative disc to determine the drug sensitivity and resistance pattern of the isolates. After incubation for 24 hours at 37°C, the isolates were considered sensitive by measuring the zone of inhibition which was compared with the zone diameter interpretative chart (NCCLs, 2007).

RESULT

The appearance and microscopy of the urine collected are seen in table 1 and table 2 shows the various biochemical test carried out on the isolates as well as gram stain, shape and morphology of the identified organisms. Table 3 shows that from the 91 isolates, *Staphylococcus aureus* had the highest growth with 26 (28.6%) followed by *Klebsiella spp* 25 (27.5%) and *Escherichia coli* 18 (19.8%). *Pseudomonas spp* had the least growth 1 (1.1%) followed by *Proteus spp* 2 (2.2%) with *CoN Staphylococcus* and *Streptococcus spp* with 3 (3.3%) growth respectively. Diptheriod had growth of 7 (7.7%) and *Candida spp* had a growth of 6 (6.6%).

In table 4, the male and female ratio were compared, however there was a higher incidence of UTI in female (70.6%) than male (29.6%). Table 5, shows the bacteria pathogen isolated in relation to different age groups. Of the 78 samples that yielded significant bacterial growth, the highest number of isolates 69 (88.5%) was found among age group of 21 years-above followed by children between 0-10 years with 6 (7.7%) and the least at 3 (3.9%) at age group of 11-19.

Table 6, shows that all gram positive bacteria showed resistance to coxacillin and sparfloxacin. *Staphylococcus aureus* showed varying susceptibility to Gentamycin (69.2%), Streptomycin (42.6%), Ciprofloxacin (50.0%), Amoxicillin(23.1%) and Perfloxacin(19.2%). *CoNS* showed resistance to Sparfloxacin, ampiclox, Ceftazidine and Coxacillin with perfloxacin sensitive. *Strptococcuspp* was sensitive to gentamycin and ciprofloxacin (100%) followed by perfloxacin and ampiclox(66.7%). Table 7 shows that all gram negative organisms were sensitive to streptomycin except *Klebsiellaspp* which some were resistant. *Escherichiacoli* was the highly sensitive to streptomycin(88.9%) and gentamycin (66.7%) followed by tarvid(55.6%). It showed varying resistance to septrin(44.4%) and Ciprofloxacin 38.9%, *Pseudomonasspp* shows sensitivity to septrin, chloramphenicol, gentamycin and perfloxacin(100%). *Proteus spp* showed resistance to sparfloxacin, perfloxacin and tarvid(100%) and was mildly sensitive to septrine chloramphenicol, ciprofloxacin, amoxicillin, augumentim and gentamycin (50%) and was sensitive to only streptomycin (100%). *Klebsiellaspp* showed varying sensitivity to gentamycin (76.0%), tarvid(52.0%) and streptomycin (24.0%). It showed varying resistance to ciprofloxacin 24.0% and augumentin(52.0%).

Table 1: Shows the appearance and microscopy of urine collected.

Urine Appearance	Microscopy
Yellow	Epithelial cells, Sperm cells
Deep yellow	Pus cells, Amorphous phosphate
Amber	Yeast cells, Hyaline cast
Pale Amber	Calcium oxalate, Epithelial cells
Cloudy and Amber	Yeast cells, Calcium oxalate.

Table 2: Shows the morphological and identification of the isolates.

Isolates	Morphology	Gram stain	Shapes	Catalase	Coagulase	Indole	Methyl red	Citrate
<i>Staphylococcus aureus</i>	Deep yellow colonies, uniform in colour	+ve	Cocci	+ve	-ve	-ve	+ve	+ve
<i>Klebsiellaspp</i>	Yellow to whitish-blue colonies, extremely mucoid	-ve	Rod	+ve	-ve	-ve	-ve	+ve
<i>Escherichia coli</i>	Opaque yellow colonies with deep yellow centers	-ve	Rod	+ve	-ve	+ve	+ve	-ve
<i>Diphtheriodspp</i>	Gray colonies	+ve	Rod					
<i>Candidaspp</i>		+ve	Cocci					
<i>CoNStaphylococcus</i>	Pale yellow colonies	+ve	Cocci,	+ve	-ve	-ve	+ve	+ve
<i>Streptococcusspp</i>	Opaque gray-green colonies	+ve	cocci	-ve	-ve	-ve	-ve	-ve
<i>Proteusspp</i>	Translucent blue colonies	-ve	Rod	+ve	-ve	-ve	-ve	+ve
<i>Pseudomonasspp</i>	Green colonies with	-ve	Rod	+ve	-ve	-ve	-ve	+ve

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-ve = negative
 +ve = positive

Table 3: Shows the frequency of isolates from urine sample.

Isolate	Numbers (%)
<i>Staphylococcus aureus</i>	26 (28.6%)
<i>Klebsiella spp</i>	25 (27.5%)
<i>Escherichia coli</i>	18 (19.8%)
Diphtheriod	7 (7.7%)
<i>Candida spp</i>	6 (6.6%)
CoN <i>Staphylococcus</i>	3 (3.3%)
<i>Streptococcus spp</i>	3 (3.3%)
<i>Proteus spp</i>	2 (2.2%)
<i>Pseudomonas spp</i>	1 (1.1%)
Total	91 (100%)

Key: CoNS = Coagulase Negative *Staphylococcus*

Table 4: Frequency of bacteria isolated from urine sample and their relation to sex

Isolate	Sex		
	Male (%)	Female (%)	Total (%)
<i>Klebsiella spp</i>	8 (10.3)	17 (21.8)	25 (32.1)
<i>Escherichia coli</i>	5 (6.6)	13 (16.7)	18 (23.1)
<i>Proteus spp</i>	2 (2.6)	0	2 (2.6)
<i>Pseudomonas</i>	0	1 (1.3)	1 (1.3)
<i>Staphylococcus aureus</i>	5 (6.4)	21 (26.9)	26 (33.3)
CoN <i>Staphylococcus</i>	1 (1.3)	2 (2.6)	3 (3.9)
<i>Streptococcus spp</i>	2 (2.6)	1 (1.3)	3 (3.9)
Total	23 (29.6)	55 (70.6)	78 (100)

Table 5: Showing prevalence among age group.

Isolate	Age			Total
	0-10	11-20	21-above	
<i>Klebsiella spp</i>	2	2	21	25
<i>Escherichia coli</i>	2	0	16	18
<i>Proteus spp</i>	2	0	0	2
<i>Pseudomonas</i>	0	0	1	1
<i>Staphylococcus aureus</i>	0	1	25	26
<i>CoNS</i> <i>Staphylococcus</i>	0	0	3	3
<i>Streptococcus spp</i>	0	0	3	3
Total	6 (7.7%)	3 (3.9%)	69(88.5%)	78

Table 6: Antibiotics susceptibility pattern of gram positive isolate.

Isolate		CN	CPX	SP	PEF	APX	AM	S	CAZ	R	CXC(%)
<i>S. aureus</i> N=26	S	18(69.2)	13(50.0)	0	5(19.2)	9(34.6)	6(23.1)	12(42.6)	3(11.5)	4(15.4)	0
	R	4(15.4)	5(19.2)	4(15.4)	1(3.5)	7(26.9)	7(26.9)	2(7.7)	3(11.15)	3(11.15)	9(34.6)
CoNS N=3	S	2(66.7)	2(66.7)	0	3(100)	0	2(66.7)	1(33.1)	0	1(33.1)	0
	R	1(33.3)	1(33.3)	3(100)	0	2(66.7)	1(33.3)	2(66.7)	3(100)	0	3(100)
<i>Strep. spp</i> N=3	S	3(100)	3(100)	0	2(66.7)	2(66.7)	1(33.3)	0	1(33.3)	1(33.3)	0
	R	0	0	3(100)	1(33.3)	1(33.3)	0	3(100)	0	2(66.7)	2(66.7)

CN= Gentamycin, CPX= Ciprofloxacin, SP= Sparfloxacin, PEF= Perfloxacin, APX= ampiclox, S= Streptomycin, AM=Amoxacillin, CAZ= Cefcazidine, R= Recephin, CXC= Coxacillin.

S= Sensitive

R= Resistant

CoNS= Coagulase Negative Staphylococcus

Table 7: Antibiotic susceptibility pattern of gram negative isolate.

Isolate		SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S(%)
<i>E. Coli</i> N=18	S	2(11.1)	5(27.8)	2(11.1)	10(55.6)	2(11.1)	3(16.7)	12(66.7)	6(33.3)	10(55.6)	16(38.9)
	R	8(44.4)	3(16.7)	4(22.2)	7(38.9)	4(32.2)	12(66.7)	3(16.7)	5(27.8)	4(2.2)	0
<i>Pseudomonas</i> N=1	S	1(100)	1(100)	0	0	0	0	1(100)	1(100)	0	1(100)
	R	0	0	1(100)	1(100)	1(100)	1(100)	0	0	0	1(100)

<i>Proteus</i>	S	1(50)	1(50)	0	1(50)	1(50)	1(50)	1(50)	0	0	2(100)
N=2	R	1(50)	1(50)	2(100)	1(5)	1(50)	1(50)	1(50)	2(100)	2(100)	0
<i>Klebsiella</i>	S	1(4.0)	3(12.0)	2(8.0)	3(12.0)	2(8.0)	2(8.0)	19(76.0)	3(12.0)	13(52.0)	6(24.0)
N=25	R	6(24.0)	2(8.0)	4(16.0)	6(24.0)	3(12.0)	13(52.0)	4(16.0)	3(12.0)	5(20.0)	1(4.0)

SXT= Septrin, CH= chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxicillin, AU= Augumencin, CN= Gentamycin, PEF= Perfloxacin, OFX= Tarvid, S=Streptomycin

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The isolated organisms in this study showed that *Staphylococcus aureus* were the most common urinary tract infection pathogens isolated followed by *Klebsiella spp* and then *Escherichia coli*. Also, the result showed that females 55 (70.6%) were more infected than males 23 (29.6%) with UTIs. This implies that women are at higher risk to UTIs than males. More so, the indiscriminate use of antibiotics kills or reduce the number of the normal flora of the vagina thereby giving them more room for pathogenic bacteria to grow (Hooton *et al.*, 1996). Also, the antibacterial properties of the prostatic fluid plays a preventive role in males (Roland *et al.*, 1992). The pathogens identified in the study is similar to those of many other countries either within this region or internationally (Astal *et al.*, 2000). In this study, *Staphylococcus aureus* 26(28.6%) was the highest cause of UTI followed by *Klebsiella spp* 25(27.5%). In comparison with Ochada *et al.* 2014 report, *Escherichia coli* 19(21.6%) was the most isolated organism followed by *Klebsiella spp* 14(15.9%). This difference in frequency could be as a result of different environmental conditions and host factor such as health care, educational programs and hygiene practices in each country (Amin *et al.*, 2009).

Most of the gram positive cocci in this study showed sensitivity to ciprofloxacin, gentamycin and resistance to coxacillin and sparfloxacin. In comparison with Ochada *et al.*, (2014). *Staphylococcus aureus* were more sensitive to augumentin and erythromycin and resistance to amoxicillin and coxacillin.

The enterobacteriaceae family were the most common microorganisms isolated from urinary tract infection in this study with (50.6%) which is similar to the work of Amin *et al.*, 2009 who reported 54.4% of enterobacteriaceae isolated.

Klebsiella spp was the most isolated gram negative organism and was more in females (31.8%) than in males (10.3%) but according to Amin *et al.*, (2009) report, *Escherichia coli* was the most isolated organisms and was also more in females (75.5%) than in males (24.5%).

Resistance of the isolates to some antibiotics such as augumentin, ampicillin and coxacillin agrees with the report of Ochada *et al.*, 2014 who reported resistance of most of its isolate to augumentin, ampicillin and coxacillin.

Conclusion

Antibiotics resistance is an important public health concern now. Antibiotic sensitivity pattern of organisms is changing rapidly in developing countries due to misuse of antibiotics. Resistance to most of these microbial agents by the isolate can also be due to indiscriminate use of these agents by physicians and patients.

Recommendation

It is advised that patient should avoid self-prescription and there should be strict control of antibiotics which might help reverse this situation.

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