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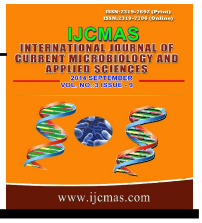

Original Research Article

Sensitivity profile of *Staphylococcus aureus* isolates obtained from patients with urinary tract infection in Kaduna Metropolis, Kaduna, Nigeria

Nandita De[1]* and Stanley Pius Ishaya[2]

[1]Department of Biological Sciences, Covenant University, Ota, Nigeria
[2]Department of Microbiology, Kaduna State University, Kaduna, Nigeria
**Corresponding author*

A B S T R A C T

One hundred and one (101) urine samples were collected from patients with complications of UTI attending different hospitals in Kaduna Metropolis, Kaduna. Out of one hundred and one urine samples collected and analyzed, forty three were from male patients and fifty eight were from female patients. When all these urine samples were cultured on Mannitol salt agar, fifty eight isolates were obtained from the samples and all these isolates were gram positive cocci. Out of fifty eight isolates, twenty three isolates showed yellow color on the medium surrounding the growth in MSA and these were also catalase and coagulase positive. All these isolates showed hemolysis on blood agar medium. Susceptibility test indicates that the isolates were highly sensitive to ciprofloxacin, gentamicin, nitrofurantoin and cotrimoxazole. There was no measurable zone of inhibition for two antibiotics, augmentin and amoxicillin, against these 23 isolates. Out of 23 isolates, sixteen were isolated from urine samples which were cloudy in nature. Out of these 23 isolates, seventeen were isolated from urine samples of female patients. ANOVA shows (F= 34.39) that there is a significant difference in diameters of zones of inhibition of four antibiotics against the isolates.

Keywords

Hemolysis, Sensitivity test, Catalase, Coagulase, Ciprofloxacin

Introduction

Gram-positive organisms like *Staphylococcus saprophyticus* (causative organism in 5% to 15% of UTIs), *Enterococcus faecalis;* Gram-negative organisms like *Escherichia coli* (causative organism in 85% of community-acquired infections), *Klebsiella pneumonia, Proteus* species, *Pseudomonas aeruginosa, Enterobacter* and *Serratia* species are associated with UTIs. Rare causes include *Salmonella* species, Mycobacterium *tuberculosis, Chlamydia trachomatis, Candida* species (more common in immune compromised patients, patients with diabetes, and patients who have recently received antibiotics) and *Staphylococcus aureus*. Presence of *S. aureus* results from either a primary (ascending) UTI or as a consequence of bacteremia with secondary spread to the kidneys (Jensen *et al*., 2002). Studies have shown that 5.5% to 8.3% of patients with staphylococcal UTI who go

untreated develop secondary bacteremia. While a primary *S. aureus* UTI simply requires a course of oral antibiotics, patients with secondary bacteremia require intravenous antibiotics and multiple investigations to rule out secondary complications which include endocarditis, osteomyelitis and septic shocks. In order to prevent *Staphylococcal* UTI, it is extremely important to have the proper antibiotics. The initial choice of drug depends on patient's history (e.g. recurrent or frequent infections, recent antibiotic therapy) and the prevalence of resistant organisms in the community (Weems, 2001).

Drugs commonly recommended for simple UTIs include sulfamethoxazole, trimethoprim, amoxicillin, nitrofurantoin, ampicillin, ciprofloxacin and levofloxacin. Effective treatment of gram positive blood stream infection (bacteremia), including those caused by *Staphylococcus*, *Streptococcus*, and *Enterococcus* species, represented major clinical challenge. *Staphylococcus aureus* bloodstream infection is among the most prevalent and difficult to treat. This incidence of *S. aureus* bacteremia (SAB), particularly bacteremia caused by methicillin-resistant *S. aureus* (MRSA) strains, has increased dramatically in recent years in the United States and in some European countries (Shorr *et al.*, 2006). *Staphlococcus aureus* bacteriuria is associated with a high mortality rate and places a substantial cost and resource burden on health care systems. This burden is increased by the high likelihood that life-threatening complication of *Staphylococcus aureus* bacteriuria will occur, including infective endocarditis and metastatic infections (Fowler *et al.*, 2005).

Resistance of *S. aureus* strain to antibiotics has been increasing; thus the ability of this pathogen to spread in both hospitals and community settings has increased. Increased antibiotic resistance in addition to the increased frequency of invasive surgery, increased uses of intravascular devices and increased member of patients with immune compromised status because of HIV infection or immune suppression after transplantation or cancer treatment, has led to sharp increases in the incidence of SAB over the past 30 years (Miro *et al.*, 2005). Some strains of *S. aureus* are capable of producing staphyloxanthin-a membrane bound carotenoid and it has been suggested that staphyloxanthin can protect *S. aureus* against oxidative stress. Staphyloxanthin can be regarded as a biological antioxidant against hydrogen peroxide and hydroxyl radicals and might be useful as a therapeutic radical scavenger that acts as a virulent factor (Clauditz *et al.*, 2006). In the present era, effective control of this disease agent is compromised by rapid evolution of antimicrobial resistance in both community and hospital settings. In principle, the inhibition of carotenogenesis may offer a novel therapeutic approach to the treatment of complicated *S. aureus* infections, effectively rendering the pathogen more susceptible to clearance by normal host innate defenses (Liu *et al.*, 2005).

However, the resistance ability of *S. aureus* increases due to the widespread uses of these antibiotics. Methicillin-resistant *S. aureus* strains carry the mecA gene that encodes PBP2A, the central determinant of methicillin-resistance, and is carried by a mobile genetic element designated staphylococcal cassette chromosomal mec (SCC mec) (Ito *et al.*, 2003). The MRSA contains the ability of resistance of macrolides, aminoglycoside, lincosamide, tetracycline and other antimicrobial drugs. It has been shown that colonization and infection of MRSA in healthy people has increased. MRSA can transmit between

person to person, and human and animals, air transmission and contaminated inanimate object to living things. SCC mec types I, II, III and VI have been mostly linked to health care associated MRSA strains (HA-MRSA) while types IV and V have been commonly associated with community associated isolates (CA-MRSA) (Feng *et al*., 2008). An investigation was made here on the sensitivity profile of *S. aureus* isolates obtained from patients suffering from UTI against some commonly prescribed antibiotics.

Materials and Methods

Study area

The selected study area was Kaduna metropolis, Kaduna. The National Petroleum Cooperation hospital (NPCH) located at Kachia road, Kaduna and Nursing home (NH) located opposite ASSA pyramid hotel, Kaduna was selected for this purpose. One hundred and one (101) samples were collected from patients complaining of urinary tract infection. Forty three samples were collected from male patients whereas fifty three were collected from female patients. The appearance and pH value of each urine sample was also recorded. The study period was between the months of July 13 to August 13.

Collection of samples

The urine samples were collected from the patients with the help of laboratory staff numbers of hospitals. The aim of the research work was explained to them before collection of urine samples. Each patient was given a sterile wide-necked, leak proof container. Explanations were made to the patients to collect clean catch midstream samples. After collection and labeling, specimens were sent to the laboratory for immediate processing.

Processing of collected specimens:

A loopful of each of urine samples was inoculated on CLED agar and Mannitol salt agar (MSA) and the plates were incubated at 37^{o}C for 24 h. The round and white to yellow colonies of 1–3 mm in diameter were isolated and labeled. These were subcultured on blood agar for observation of hemolysis on blood agar (BA). The colonies which showed wide zone of clear hemolysis were collected and labeled and were kept in refrigerator for identification purpose.

Identification of *Staphylococcus aureus:*

(a) Morphological and cultural characteristics: The appearance and color of the colonies on CLED agar, MSA agar and Blood agar and diameter of the colonies were noted. Gram staining was done following the procedure as described in Benson (2005).

(b) Biochemical characterization of the isolates: The isolates were also identified using different biochemical tests like catalase test, coagulase test, sugar utilization tests. Isolates were then tested for coagulase production using Staphytect Plus test method. The reagents were purchased from Sanofi Diagnostic Pasteur, France and the method was followed as instructed by manufacturer.

Sensitivity profile of the isolated *S. aureus* strains

All 23 isolates were used for this test using streak plate method. Sensitivity disks containing conventional antibiotics like augmentin (20 µg), Amoxicillin (10 µg), ciprofloxacin (5 µg), cotrimoxazole (30 µg), gentamicin (10 µg) and nitrofurantoin (300 µg) manufactured by BIOTECH LABS., England were used for sensitivity test. A

loopful of growth of each isolate on mannitol salt agar was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of $1x10^8$ cells/ml) before inoculation. Mueller-Hinton agar was inoculated with 0.5 ml suspension of each isolate adjusted to $1x10^8$ cells/ml using sterile spreader. Sensitivity discs containing antibiotics were placed on the surface of each Mueller-Hinton agar plate evenly seeded with test organisms and was incubated for 24 h at 37^oC. The isolates showing the zone size of 20 mm or less for ciprofloxacin, 14 mm or less for nitrofurantoin, 12 mm or less for gentamicin and cortimixazole and 19 mm or less were considered as resistant strains for these antibiotics (CLSI, 2007).

ANOVA test was done in order to see the difference in diameters of zones of inhibition of the antibiotics against the isolates is significant or not.

Results and Discussion

Description of samples

A total number of one hundred and one urine samples were collected from patients attending the two hospitals. Out of one hundred and one urine samples analyzed, forty three (42.6%) were from male patients and fifty eight (57.4%) were from female patients. Thirty four (34) of these samples were cloudy in nature and the pH values of the samples were in the range of 5.0–9.0. The range of age of the patients was 5–59 years. Twenty two (21.8%) samples were collected from patients of the age group 0f 1–20 years whereas 63 (62.4%) were collected from patients of 21–40 years age group and 16 (15.8%) were collected from patients of age group of 41–60 years (Table 1).

Isolation and identification of isolates:

When all these urine samples were cultured on Mannitol salt agar, fifty eight isolates were obtained from 58 urine samples (57.4%) and all these isolates obtained were gram positive cocci. Out of fifty eight isolates, twenty three isolates (22.8%) showed yellow color on the medium surrounding the growth in MSA and these were also catalase and coagulase positive. These twenty three isolates also showed hemolysis on blood agar medium. The results are expressed in Table 2.

Out of 23 isolates, 16 were isolated from urine samples which were cloudy in nature. Out of 23 isolates, 17 were isolated from urine samples of female patients (Table 3).

Antibiotic susceptibility test

Susceptibility test indicates that the isolates were highly sensitive to ciprofloxacin (100%), gentamicin (100%), nitrofurantoin (100%) and cotrmoxazole (83%) . There was no measurable zone of inhibition for two antibiotics, augmentin and amoxicillin, against these 23 isolates. The results are shown in Table 4. ANOVA shows (F= 34.39) that there is a significant difference in diameters of zones of inhibition of four antibiotics against the isolates (Table 5).

From this study it has been observed that out of 101 samples, 23 isolates (22.8%) of *S. aureus* were obtained from 23 urine samples. This agrees with the findings of Akerele *et al.,* (2000) who reported a recovery rate of 35.6% *S. aureus* in Benin-city, Nigeria. Out of 23 isolates, 17 isolates were obtained from female patients (73.91%) which support the work of Akortha and Ibadin (2008) and Mordi and Erah (2006). This may be due to the fact that a relatively short urethra in women which

makes it subject to fecal contamination and colonization with potentially pathogenic bacteria that has only a few centimeters to traverse to the bladder. Out of 17 *S. aureus* infected female patients, 14 (82.35%) were in the range of 16–35 yrs of age. This observation supports previous findings (Nwanze *et al*., 2007; Akortha and Ibadin, 2008) and may be due to sexual intercourse and use of a diaphragm for contraception. This suggests that sexually active women are at highest risk of community-acquired UTIs (Manges *et al*., 2008).

Out of 23 patients who were infected with *S. sureus*, 6 were male patients suffering from UTI. Though it is not common to have UTI among male below 50 yrs old, the age range of three male patients having UTI *S. aureus* infection was 20–37. This may due to the relationship between men and women which usually favors the transfer of this organism leading to the increasing level of prevalence among the men. Out of 23 isolates, 16 isolates were obtained from urine samples which were cloudy in nature. The urine samples are cloudy or bloody in nature if patients are suffering from bladder infection (cystitis) (Gupta *et al*., 2011).

The susceptibility test results of *S. aureus* in the study showed 100% resistance to augmentin and amoxicillin, and 100% sensitivity to ciprofloxacin, gentamicin, nitrofurantoin and 87% sensitivity to cotrimoxazole. Thus, indicating that all the *S. aureus* isolates involved in UTIs were augmentin and amoxicillin resistant and 13% of them showed very high resistance to cotrimoxazole. The organism's resistance to these agents might be due to the easy availability of the antimicrobial agents in this environment which usually leads to their frequent and indiscriminate use in UTIs (Okeke *et al*., 1999).

Table.1 Distribution of samples based on sex and age

Age	Male	Female	Total (%)
1–20	8	14	22 (21.8)
21–40	25	38	63 (62.4)
41–60	11	5	16 (15.8)

Table.2 Morphological and biochemical characteristics of isolates

IN	GR	MSA (medium turns to yellow)	BA (hemolysis)	Catalase	Coagulase	Organism
A1-A23	Gram +ve cocci	+	+	+	+	*S. aureus* in clusters
A 24-A58	”	-	-	+	-	*Staphylococcus* sp.

Key: GR-gram reaction

Table.3 Distribution of *S. aureus* among male and female patients

S/N	Sample Code	Sex	Age	Appearance
1	4	Female	29	Cloudy
2	8	"	31	Brown and cloudy
3	11	"	32	"
4	15	"	21	Cloudy
5	19	"	35	Red and cloudy
6	26	"	22	Yellow-brown
7	28	Male	20	Brown and cloudy
8	32	"	48	"
9	39	"	49	Cloudy
10	43	Female	21	Brown and cloudy
11	45	Male	32	Red and cloudy
12	52	Female	16	Cloudy
13	57	"	24	Red and cloudy
14	69	"	30	Cloudy
15	71	Male	45	Yellow-brown
16	73	"	27	Milky white
17	77	Female	30	Red and cloudy
18	80	"	37	Yellow-brown
19	83	"	41	Cloudy
20	86	"	23	Yellow-brown
21	91	"	21	Cloudy
22	95	"	45	Yellow-brown
23	100	"	31	"

Table.4 Sensitivity profile of isolates against different antibiotics

S/N	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	4. 0	4.0	4.0	4.5	3.9	4.9	3.7	3.8	4.0	3.8	3.7	3.7	3.5	3.8	3.5	4.9	4.7	3.6	4.0	4.1	4.4	5.1	4.5
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2.9	1.9	1.6	3.5	3.7	2.3	2.6	2.8	2.4	2.2	2.6	2.3	2.5	2.7	2.3	2.7	2.3	2.3	2.4	2.0	2.6	2.9	2.6
4	2.4	2.9	1.9	2.3	3.1	2.5	2.6	1.9	2.8	2.3	2.9	2.5	2.5	2.8	2.4	2.5	2.9	2.5	2.8	2.5	3.4	3.0	3.2
5	2.6	2.8	2.3	2.7	2.7	2.4	0.4	0.6	3.5	2.2	3.8	3.2	2.5	2.6	2.2	3.4	3.8	0.4	0.5	2.2	2.5	3.4	3.0
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: 1→ ciprofloxacin (10µg/ml) A-W→ isolates
2→ augmentin (30µg/ml)
3→ gentamicin (10µg/ml)
4→nitrofurantoin (200µg/ml)
5→ cotrtimoxazole (25µg/ml)
6→ amoxicillin (25µg/ml)

Table.5 Showing Biostatistical test for four antibiotics against the isolates

S/N	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	4.0	4.0	4.0	4.5	3.9	4.9	3.7	3.8	4.0	3.8	3.7	3.7	3.5	3.8	3.5	4.9	4.7	3.6	4.0	4.1	4.4	5.1	4.5
3	2.9	1.9	1.6	3.5	3.7	2.3	2.6	2.8	2.4	2.2	2.6	2.3	2.5	2.7	2.3	2.7	2.3	2.3	2.4	2.0	2.6	2.9	3.2
4	2.4	2.9	1.9	2.3	3.1	2.5	2.6	1.9	2.8	2.3	2.9	2.5	2.5	2.8	2.4	2.5	2.9	2.5	2.8	2.5	3.4	3.0	3.2
5	2.6	2.8	2.3	2.7	2.7	2.4	0.4	0.6	3.5	2.2	3.8	3.2	2.5	2.6	2.2	3.4	3.8	0.4	0.5	2.2	2.5	3.4	3.0

F_{obs}= 34.39

$F_{3, 88, 0.95}$= 2.67

Since F_{obs} value is greater than F_{cal} value, we can say that there is a significant difference between the diameters of zones of inhibition of the isolates against four antibiotics.

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