RESISTANCE PATTERN OF UROPATHOGENIC STAPHYLOCOCCAL STRAINS ISOLATED FROM OUTPATIENTS IN A NIGERIAN HOSPITAL

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ABSTRACT

Bacterial resistance to antibiotics is a global problem which results in difficulty in treatment. This study determined antimicrobial resistance of staphylococci isolated from urogenital tracts of humans with a presumptive diagnosis of urinary tract infection (UTI). Forty five urogenital samples (endocervical swab, high vaginal swab and urine) were obtained from outpatients at Igbinedion University Teaching Hospital between April and May, 2010. They were processed for isolation of *Staphylococcus* sp. Colonies in pure culture were identified by biochemical reactions and tested for susceptibility to 9 antimicrobials using disk agar diffusion method. Minimum Inhibitory Concentration (MIC) was determined by macrodilution method. Ten isolates of staphylococci were obtained (22% of the total samples). *Staphylococcus aureus* was the most frequent species (70%). All the isolates were multidrug resistant with each isolate exhibiting resistance to at least 5 antimicrobials. All the isolates had 100% resistance to nitrofurantoin, ampicillin, ciprofloxacin, augmentin, and ceftriazone. All coagulase negative *Staphylococcus* sp. strains were susceptible to doxycycline while *S. aureus* strains were relatively susceptible to TMP/SMX. The study reports the alarming antimicrobial resistance of members of the *Staphylococcus* genus isolated from human urogenital tract. There should be strict control in the use of antibiotics for chemotherapy of staphylococcal infections to reduce the organism's resistance to commonly used antibiotics.

Keywords:-Resistance, antibiotics, uropathogens, multidrug resistance.

INTRODUCTION

Staphylococcus aureus are bacterial strains that commonly colonize the skin and the anterior nostrils of healthy people. In fact, 20% to 30% of all individuals are colonized with staphylococci. [1]. Staphylococci, besides being commensals on mucosal surfaces and skin, are often implicated in a variety of infections, e.g. abscesses, furuncles, pyoderma, pneumonia, and bacteremia [2]. *Staphylococcus aureus* has long been responsible for a great deal of human morbidity and mortality throughout history. It is also one of the causative agents of urinary tract infections. Urinary tract infections (UTI) are common infectious diseases that can be associated with substantial morbidity and significant expenditures. Worldwide, it is estimated that several hundred millions women suffer from UTI annually with a recurrence rate of between 27% and 48% [3]. The annual cost to health care services is staggering, reaching $2 billion in the United States alone and over $6 billion worldwide [3] *Escherichla coli* is the causative agent in most cases (up to 85%) followed by staphylococci*, Klebsiella pneumoniae* and enterococci *[4]*. Bacterial urinary tract infections (UTI) are a major clinical problem in human and are among the most common indications for antimicrobial therapy. However, *S. aureus* has had significant resistance changes over the years and this has important clinical and therapeutic implications. Soon after the introduction of penicillin in 1941, resistant strains of *S. aureus* emerged, first in hospital settings and then disseminated in the community [5]. Today, most *S. aureus* strains are resistant to penicillin. *S. aureus* resistance to other penicillins and cephalosporins have been undergoing a similar process as more and more strains have acquired the mecA gene that encodes penicillin-binding protein 2A [6]. The recent increased recognition of health implications of multiple drug resistant *S. aureus* in treatment of infections has important clinical and pharmacological implications for the health care provider. Since members of this genus have a high frequency of conjugation and frequently acquire plasmids that encode antimicrobial resistance [7], constant surveillance for resistance to antimicrobials is required.

The purpose of this study was to evaluate the antimicrobial resistance of isolates of staphylococci obtained from human urogenital tracts with a presumptive diagnosis of UTI.

MATERIALS AND METHODS

Collection and analysis of samples

Forty five samples comprising of 15 Endocervical Swab (ECS), 15 High Vaginal Swab (HVS) and 15 urine samples were collected from outpatients at Igbinedion University Teaching Hospital, Okada, Nigeria by the staff of Medical Microbiology unit of the hospital between April and May, 2010. The samples were microbiologically examined for the presence of staphylococcal strains by inoculating on McConkey agar (Lab M, UK) media plates. Plates were incubated at 37^0C for 24 hrs. Isolates were examined for colonial morphology and lactose fermenting ability to exclude lactose fermenters. Non lactose fermenters were selected and Gram stained. Direct microscopic examination of Gram stained slides were done using saline solution with ×10 and ×40 objectives microscope. Gram positive cocci with grape bunch morphology were selected.

Identification of Staphylococcus aureus strains

The following tests were done to confirm the identities of the isolates.

Catalase Test

Catalase test was done by adding a drop of 3% H_2O_2 to a slide, by means of a loop wire, an innoculum of 18 hr old culture of each organism was made on the drop. Production of effervescence indicates a positive result for *Staphylococcus* species and distinguish them from *Streptococcus* species which is also Gram positive cocci.

Coagulase Test: 0.5 ml of reconstituted lyophilized rabbit coagulase plasma was added to EDTA in a sterile tube. A colony of suspected *Staphylococcus* sp. was innoculated into the tube containing rabbit plasma, and mixed thoroughly. The tube was incubated at 35^0C for 6 hours. The tubes were observed for the presence of clotting which is a positive reaction for *S. aureus*.

Growth on Mannitol salt agar

The suspected *Staphylococcus* sp. strains were streaked on Mannitol salt agar plates. Plates were incubated at 37ºC for 18-24 hrs aerobically. Growth of colonies with change in colour of the media from red to yellow confirms the presence of *S. aureus*.

Antibiotic susceptibility tests

The susceptibility of the bacteria to different antibiotics was tested according to a breakpoint method which agrees with standard indicated by National Committee for Clinical Laboratory Standard (NCCLS, USA), using standard antibiotic discs (Oxoid, UK). Eighteen hours old broth culture of *Staphylococcus* sp. strains of approximately 10^8 cfu1ml which is equivalents to 0.5 MacFarland standard was inoculated onto solidified and sterilized Muller Hinton agar by spread plate method. Nine different standard antibiotic disks namely: Nitofurantoin (300 µg), Ampicilin (10 µg), Augmentin (50 µg), Ceftriazone (35 µg), Ciprofloxacin (5 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg) and Cotrimoxazole (Trimethoprim/Sulphamethoxazole TMP/SMX (1.25/23.75 µg)) were placed firmly at least 2 cm apart on the agar plates using a sterile forceps. The plates were kept on the bench for 30 min to allow diffusion of the antimicrobials. The plates were then incubated for 24 hr at 37^oC and examined for clear zones of inhibition around the discs. The diameter of inhibition were measured and compared with a standard antibiotic resistance chart zones to determine susceptibility or resistance of the strains as instructed by the manufacturer of the antibiotic discs (Oxoid, UK).

Determination of Minimum Inhibitory Concentration (MIC)

All isolates that shows gross resistance to ampicilin, augmentin and ceftriazone were assayed for their MIC to the antibiotics. Stock solution of Ampicillin (Bal Pharma, China), Augmentin and Ceftriazone were prepared to concentration of 100 µg/ml, 400 µg/ml and 300 µg/ml respectively. The MIC of the antibiotics to the bacterial strains was determined by adding 1 ml of stock antibiotic solution to 9 ml of nutrient broth thereby reducing the concentration by 1/10. Five millimeter of the medium/antibiotics solution was added to another 5 ml of nutrient broth thereby halving the antibiotic concentration. This was repeated until the least desired antibiotic concentration was obtained. 100 µl of 18 hr old staphylococcal culture of approximately 10^8 cfu/ml which is equivalent to McFarland standard 0.5 was inoculated into each antibiotics/medium mixture and incubated at 37°C for 24 hr. The tubes were examined for growth. The least concentration at which no bacterial growth can be discerned is selected as the MIC.

RESULTS

Seven *S. aureus* and 3 *Staphylococcus* sp. strains were isolated from urogenital tract samples comprising four urine samples, three HVS and three ECS. (Table I). The identified *Staphylococcus* sp. strains were all Gram positive and catalase positive. They all grew on Mannitol Salt Agar. The *S. aureus* strains grew on MSA with yellow colonies and were coagulase positive while *Staphylococcus* sp. strains grew on MSA with pink colonies and were coagulase negative. (Table I). These characteristics confirm the identities of the *Staphylococcus* species. Antibiotic susceptibility tests were performed on all *Staphylococcus* sp. strains by disc diffusion method. All coagulase negative *Staphylococcus* sp. strains were susceptible to doxycycline while highly resistant to other tested antibiotics. The *S. aureus* strains were highly resistant to tested antibiotics. The lowest resistance value (57%) was observed in TMP/SMX (Table II). Generally, all tested *Staphylococcus* sp. strains had 100% resistance to nitrofurantoin, ampicillin, ciprofloxacin, augmentin, and ceftriazone. 70% resistance was observed to TMP/SMX while 80% resistance was observed to chlorapheniucol and gentamicin respectively. Each strain had high overall resistance pattern that ranges between 55% and 100% to all tested antibiotics. *Staphylococcus aureus* OMG 003, 005 and 009 were resistant to all tested antibiotics while *Staph. aureus* OMG 004 had the lowest resistance rate of 55% to tested antibiotics. (Table II). The MIC of ampicillin, augmentin, and ceftriazone to all resistant *Staphylococcus* sp. strains were determined to discover the extent of resistance by broth macrodilution. All the tested strains exhibited very high resistance to the tested antibiotics with very high MIC. All tested strains had an MIC of ≥5 µg/ml to ampicillin, ≥10 µg/ml to augmentin and ≥7.5 µg/ml to ceftriazone (Table III).

Table I. Identification of *Staphylococcus* sp. strains by different chemical reactions

		Different chemical reactions for identification				
Sources of Isolation	Bacterial strains	Gram Staining	Catalase	Coagulase	Colour on MSA.	Inference
HVS	Strain OMG 001	+	+	+	Yellow	*S. aureus*
HVS	Strain OMG 002	+	+	-	Pink	*Staphylococcus* sp.
Urine	Strain OMG 003	+	+	+	Yellow	*S. aureus*
ECS	Strain OMG 004	+	+	+	Yellow	*S. aureus*
HVS	Strain OMG 005	+	+	+	Yellow	*S. aureus*
ECS	Strain OMG 006	+	+	+	Yellow	*S. aureus*
Urine	Strain OMG 007	+	+	+	Yellow	*S. aureus*
ECS	Strain OMG 008	+	+	-	Pink	*Staphylococcus* sp.
Urine	Strain OMG 009	+	+	+	Yellow	*Staph aureus*
Urine	Strain OMG 010	+	+	-	Pink	*Staphylococcus* sp.

Note: MSA means Mannitol Salt Agar

Table II. Antibiotic resistance pattern of *Staphylococcus* sp. strains.

cterial Strain	NITRO 300 µg B.P ≤.14	AMP 10 µg B.P. ≤ 15	COT 10 µg B.P. ≤10	CIP 5 µg B.P. ≤ 15	DOXY .30 µg B.P. ≤ 12	AUG 50 µg B.P. ≤ 19	CHLOR .30 µg B.P. ≤12	CTZ 35 µg B.P. ≤ 13	GEN 10 µg B.P. ≤12	% Resistance A
aureus OMG 001	10mm R	0mm R	0mm R	15mm R	9mm R	13mm R	16mm S	0mm R	15mm S	77.8
phylococcus sp. OMG 002	10mm R	0mm R	6mm R	4mm R	15mm S	5mm R	10mm R	0mm R	9mm R	88.9
aureus OMG 003	14mm R	0mm R	5mm R	10mm R	7mm R	6mm R	0mm R	0mm R	10mm R	100
aureus OMG 004	11mm R	0mm R	15mm S	10mm R	13mm S	0mm R	20mm S	0mm R	14mm S	55
aureus OMG 005	10mm R	5mm R	8mm R	5mm R	0mm R	0mm R	6mm R	5mm R	7mm R	100
aureus OMG 006	10mm R	0mm R	17mm S	15mm R	15 mm S	0mm R	0mm R	0mm R	0mm R	77.8
aureus OMG 007	10mm R	0mm R	4mm S	5mm R	7mm R	4mm R	6mm R	4mm R	6mm R	88.9
phylococcus sp OMG 008	11mm R	0mm R	0mm R	0mm R	13mm S	0mm R	6mm R	0mm R	8mm R	88.9
aureus OMG 009	12mm R	0mm R	0mm R	5mm R	5mm R	5mm R	10mm R	5mm R	12mm R	100
phylococcus sp OMG 010	12mm R	0mm R	5mm R	7mm R	14mm S	3mm R	5mm R	0mm R	8mm R	88.9
Resistance B	100	100	70	100	50	100	80	100	80	

Note:S-Susceptibility,R-Resistance

% Resistance A- Percentage resistance of each *Staphylococcus* sp. to all tested antibiotics

% Resistance B- Percentage resistance of all tested *Staphylococcus* sp strains to each antibiotic

Table III. Analysis of Minimum Inhibitory Concentration of three antibiotics to selected resistant *Staphylococcus* sp strains

Staphylococcus sp. strains	Growth of *Staphylococcus* sp strains at different antibiotic concentrations				MIC
Ceftriazone B.P. 1 µg/ml					
	30 µg/ml	15 µg/ml	7.5 µg/ml	3.75 µg/ml	
S. aureus OMG 001	-	-	+	+	15 µg/ml R
Staphylococcus sp OMG 002	-	+	+	+	30 µg/ml R
S. aureus OMG 003	+	+	+	+	> 30 µg/ml R
S. aureus OMG 004	-	-	-	+	7.5 µg/ml R
S. aureus OMG 005	-	-	+	+	15 µg/ml R
S. aureus OMG 006	+	+	+	+	> 30 µg/ml R
S. aureus OMG 007	-	-	-	+	7.5 µg/ml R
Staphylococcus sp OMG 008	-	+	+	+	30 µg/ml R
S. aureus OMG 009	-	-	-	+	7.5 µg/ml R
Staphylococcus sp OMG 010	-	+	+	+	30 µg/ml R
Ampicillin B.P. 0.06 µg/ml					
	10 µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml	
S. aureus OMG 001	-	-	+	+	5 µg/ml R
Staphylococcus sp OMG 002	-	-	-	+	2.5 µg/ml R
S. aureus OMG 003	-	+	+	+	10 µg/ml R
S. aureus OMG 004	-	-	-	+	2.5 µg/ml R
S. aureus OMG 005	-	-	-	+	2.5 µg/ml R
S. aureus OMG 006	-	-	+	+	5 µg/ml R
S. aureus OMG 007	-	+	+	+	10 µg/ml R
Staphylococcus sp OMG 008	-	-	-	+	2.5 µg/ml R
S. aureus OMG 009	-	-	+	+	5 µg/ml R
Augmentin (Amoxycillin B.P. 0.12 µg/ml Clavularic acid- Not Determined)					
	40 µg/ml	20 µg/ml	10 µg/ml	5 µg/ml	
S. aureus OMG 001	-	-	-	+	10 µg/ml R
Staphylococcus sp OMG 002	-	-	+	+	20 µg/ml R
S. aureus OMG 003	-	+	+	+	40 µg/ml R
S. aureus OMG 004	+	+	+	+	>40 µg/ml R
S. aureus OMG 005	+	+	+	+	>40 µg/ml R
S. aureus OMG 006	-	-	+	+	20 µg/ml R
S. aureus OMG 007	-	+	+	+	40 µg/ml R
Staphylococcus sp OMG 008	-	-	-	+	10 µg/ml R
S. aureus OMG 009	-	-	+	+	20 µg/ml R
Staphylococcus sp OMG 010	+	+	+	+	>40 µg/ml R

Note:-B.P. – Breakpoint, R-Resistant, I-Indeterminable. The breakpoint for MIC was adapted from breakpoint for *S. aureus* NCTC 6571 by Andrews [8]

DISCUSSION

The micro-organism responsible for Community Acquired CA-UTI in this study is *S. aureus* followed by *Staphylococcus* spp. The 10 isolates of staphylococci represent a rate of 22% of the 45 urogenital samples. Other studies in the literature cite prevalences of no more than 10% of UTI samples being positive for staphylococci [9].

Therefore, Staphylococcus sp. appears to be more prevalent in the present population than what is usually reported. Coagulase positive species were more common (70%) than Coagulase negative species (30%). This agrees with the findings of Penna *et al*, [10] who also reported the prevalence of coagulase positive staphylococci. Coagulase-negative staphylococci constitute a major component of the normal microflora of human. [11]. There was a high frequency of resistance, with each isolates being resistant to at least 5 antimicrobial and multidrug resistance being present in all the isolates from this study. This present results suggest an increasing tendency towards resistance among staphylococcal isolates from UTI. Many factors contribute to occurrence of multi-drug resistant uropathogens in Africa including misuse of antibiotics, counterfeit drugs, shortfall in infection control, public health and also, the fact that many of the resistance genetic determinants are plasmid borne, thereby providing resistance to several other classes of antibiotics and transmission to other microorganisms. Historically, *S. aureus* has mutated several times. In the 1940's, with the introduction of wide spread penicillin the pathogen was quickly controlled, but this only lasted a few years. Then resistance occurred with the production of beta-lactamase enzymes. All the Staphylococcal sp. strains had 100% resistance to the 3 β-lactam antibiotics (augmentin, ampicillin and ceftriazone) tested in this study. This may be due to the presence of β-lactamase and extended spectrum β-lactamase (ESBLs) [12, 13]

The high antibiotic resistance exhibited by the *Staphylococcus* spp used in this study to gentamicin, (an aminoglycoside) could be due to enzymatic modification of the amino or hydroxyl groups of the aminoglycosides. Also, chromosomal mutation has been demonstrated in *S. aureus* influencing transmembrane electrical potential which has been shown to produce aminoglycoside resistance [14]. The quinolones (chiefly, ciprofloxacin) are the drugs of choice for treating UTI in many regions of the world but, all the *Staphylococcus* sp. strains used in this study display an unusually high resistant rate. Adeniyi and Amajoyi [15] have also reported ciprofloxacin resistance in South West Nigeria. Green and Tillotson [16] reported that increased resistance to ciprofloxacin in developing countries could be due to an increased selective pressure caused by the use of more ciprofloxacin. This quinolone resistance could be due to chromosomally mediated mechanism that involves mutation in the drug target, over production of multidrug resistant efflux pump and the presence of plasmid-borne quinolone resistant determinant (*qnr*) [17, 18]. The resistance observed to TMP-SMX (co-trimoxazole) by some *S. aureus* strains found in this study is very disturbing. This combination is widely use for UTI in Nigeria because of it's low cost compared with other antibiotics. It is also commonly used as prophylaxis for HIV infected patients who have a high occurrence in Nigeria. This may account for a possible higher selective pressure on the agent therefore, accounting for increased rate of resistance. Huovinen *et al.[19]* observed a clear trend in the resistance to TMP-SMX with strains isolated in the developing world being more often resistant than the strains isolated in the developed countries. The clinical significance of this finding is that resistance to co-trimoxazole has been associated with concurrent resistance to other antibiotics resulting in multi-drug resistant uropathogens [20].

Multidrug resistant *Staphylococcus* sp.is an important health care problem worldwide. From this study, All coagulase negative *Staphylococcus* sp. strains were susceptible to doxycycline while highly resistant to other tested antibiotics. Likewise, the *S. aureus* strains were relatively susceptible to TMP/SMX. However, 57% resistance was observed to the antibiotics. Therefore, doxycycline can be use for treatment of urogenital infections caused by *Staphylococcus* sp. strains while the trend in this study showed no susceptible antibiotics for treatment of *S. aureus* infections although TMP/SMX can be used for susceptible strains. In spite of the fact that β-lactam antibiotics are commonly used for treatment of staphylococcal infections, the observed resistance to β-lactam antibiotics observed in this study makes this antibiotic group unsuitable for treatment of urogenital infections caused by staphylococcal strains.

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