ANTIBIOTIC RESISTANCE PATTERN OF *Staphylococcus aureus* ISOLATED FROM HIGH VAGINAL SWAB AND URETHRAL SWAB SPECIMENS

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ABSTRACT

This study was aimed at investigating the susceptibility pattern and plasmid profile of *Staphylococcus aureus* isolates obtained from high vaginal and Urethral swab using Standard Microbiological techniques. A total of fifty two samples were collected from in and out patients of Irrua Specialist Teaching Hospital Irrua. Thirty nine (75%) of the samples were from high vaginal swab while thirteen (25%) were from urethral swab. Of the fifty two samples tested, only seven (13.5%) yielded growth of *Staphylococcus aureus.* Using the disc diffusion method, all seven strains showed 100% resistance against ampicillin, amoxicillin and nalidixic acid. The highest (26.3±1.54mm) zone of inhibition recorded were reactions to ciprofloxacin. Strains SA1, SA2 and SA3 and SA4 recorded 9.5±2.82mm, 7.8±2.34mm, 7.5±2.92mm and 8.1±3.03mm mean zones of inhibition respectively to the twelve antibiotics tested. SA5, SA6 and SA7 recorded 14.3±2.93mm, 9.0±3.48mm, and 5.75±2.38mm mean zones of inhibition respectively to the twelve antibiotics tested. Mean loss of 50.0% or more of resistance marker (RM) was recorded after treatment with acridine orange and only strains SA1 and SA4 retained their resistant genes. Thus this finding suggests the need for susceptibility testing before the administration of antibiotics.

Key Words: *Staphylococcus aureus*, antibiotics resistance, antimicrobial agents.

{**Citation:** Omoigberale, M.N.O., Iyamu, M. I., Amengialue, O.O., Egharevba A. P., and Edobor, O. Antibiotic resistance pattern of *Staphylococcus aureus* isolated from high vaginal swab and urethral swab specimens. American Journal of Research Communication, 2014, 2(1): 96-107} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Antimicrobial resistance is on the rise all over the World with gradual loss of first line antimicrobials (WHO, 2012). Hence, numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance often due to selective pressure of antimicrobial usage (Oskay *et al.,* 2009). This selective pressure is the result of indiscriminate use of antibiotics and overuse of existing drugs (McGowan, 2006). Antibiotic resistance in bacteria develops either by mutation or acquisition of new genes through a process known as horizontal gene transfer. This involves the transfer of resistant genes among pathogens which are often facilitated by the localization of these genes on plasmids particularly those associated with integrons and transposons (Tenover, 2006). The last decade witnessed the emergence of *Staphylococcus aureus* as a deadly superbug. The enormous genetic plasticity of the organism assists it to endlessly evolve resistant mechanisms against existing antimicrobial agents thus necessitating the need to control the spread of resistant staphylococcal isolates in hospitals and health care settings (Gomber and Saxena, 2007). Seventy percent to ninety percent of *Staphylococcus aureus* strains demonstrate resistance to the penicillins and amino-penicillins and hence, infections are often difficult to treat because of widespread cross- resistance to aminoglycosides, macrolides, lincosamides, tetracyclines, cephalosporins, carbapenems, beta-lactamase inhibitor combinations, trimethoprim and sulphonamides. (Diekema *et al*., 2001). Soon after the use of penicillin, *Staphylococcus aureus* was found to produce beta-lactamase penicillinase. To overcome this situation, the antibiotic-methicillin was used to replace penicillin and *S. aureus* strains resistant to methicillin emerged very quickly (Woo *et al*., 2003). This same pattern was also seen following the use of vancomycin.

Curing is the process of removing plasmids from a bacterial cell and this may be observed with relaxed plasmids when the bacterial cell is grown for successive generations in the absence of a selective agent e.g. antibiotic (Trevors, 1986). The organism then becomes sensitive to the selective agent and it was initially thought that this phenomenon would profer solution in controlling the development of resistance in formerly antibiotic susceptible bacteria (Zielenkiewicz and Ceglowsk, 2001). Antibiotics such as mitomycin, rifampicin, novobiocin and flavophospholipol as well as DNA intercalating dyes such as acridine orange, ethidium bromide, acriflavine and ascorbic acid have been shown to cure many plasmids (Ramesh *et al*., 2000). Curing agents affect membrane potential, membrane permeability, protein synthesis and the processing of DNA (Viljanen and Boratynski, 1991) as well as block plasmid transfer (Zhao *et al*., 2001). Acridine orange has been shown to cure F plasmids from *Escherichia coli* and it is suggested that this dye interfers with plasmid replication, stimulating the entire plasmid loss (Salisbury *et al.*, 1972).

Treatments that increase frequency of elimination of plasmids will certainly enhance sensitivity and effectiveness of antibiotics insitu. Hence, this study is aimed at isolating *Staphylococcus aureus* from high

vaginal and urethral swab and to determine their antibiotic susceptibility patterns. It has the following objectives: (1) To determine the sensitivity profile of *Staphylococcus aureus* strains before treatment with acridine orange after incubation at 37^0C for 24 hours. (2) To determine the sensitivity profile of *Staphylococcus aureus* strains after treatment with acridine orange. (3) To determine loss of resistant markers in *Staphylococcus. aureus* strains after treatment with acridine orange.

MATERIALS AND METHODS

SPECIMEN COLLECTION

A total of 52 high vaginal swabs and Urethral swabs were collected for this study. The specimens were collected from in and out patients at the Irrua Specialist Teaching Hospital, Irrua. The specimens were collected from 13 males (Urethral swabs) and 36 females (high vaginal swabs). The specimens were collected with sterile swab sticks, sealed with adhesive tape, labelled and transported immediately to the laboratory for examination.

BACTERIOLOGICAL EXAMINATION

Swab stick containing each specimen was used to make a well of inoculum on each agar plate. The streak plate technique was used and the plates were incubated aerobically at 37^0C for 24 hours. Isolates were further identified by a series of biochemical tests.

ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiotic sensitivity testing on each of the seven isolates of *S. aureus* was carried out using the agar disc diffusion method on sterile nutrient agar plates. A loopful of each colony was picked aseptically using a flamed wire loop and placed on sterile nutrient agar plates. This was then spread all over the plates applying the caution of not touching the edges of the plates. The seeded plates were allowed to stand for about 2minutes to allow the agar surface to dry. A pair of forceps was flamed, cooled and used to pick an antibiotic multidisc. Augumentin 10µg/disc, Ciprofloxacin 10µg/disc, gentamicin 10µg/disc, ampicillin 20µg/disc, tetracycline 30µg/disc, nitrofurantoin 300µg/disc rifampicin 10µg/disc, penicillin 10µg/disc, sparfloxacin 10µg/disc, chloramphenicol 30µg/disc, nalidixic acid 30µg/disc and amoxicillin 10µg/disc. Discs were placed at least 22.0mm from each other and 14.0mm from the edge of the plate. Antibiotic discs were selected on the basis of their clinical importance and efficacy on *S. aureus.* The plates were incubated at

37^{0}C for 24hours and at the end of incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (Byron *et al*., 2003)

Treatment of *Staphylococcus aureus* strains with prepared acridine orange

The treatment of multidrug resistant (MDR) *Staphylococcus aureus* strains with acridine orange was done according to a modified method of Byron *et al*. (2003). Using a sterile pasteur pipette, 0.5ml aliquot of each diluted overnight broth culture of MDR pathogen was added to 4.5ml sterile molten nutrient agar. This was then poured on top of sterile hardened or set 2% nutrient agar plates. The same antibiotic multidiscs used before treatment were then picked (using flamed and cooled pair of forceps) and impregnated on the set agar overlay plates. Plates were incubated at 37^{0}C for 24hours. Measurement of diameters of zones of inhibition was taken and recorded (NCCLS, 2000).

RESULTS

Table 1 shows *Staphylococcus aureus* isolates SA1, SA2, SA3, SA4, SA5, SA6, and SA7 from high vaginal and urethral swab and their sensitivity reactions to ciprofloxacin, gentamicin, augmentin, chloramphenicol, ampicillin, tetracycline, amoxicillin, refapicin, penicillin, nalidixic acid, nitrofurantoin and sparfloxacin. Before treatment with dilutions of acridine orange, the seven *Staphylococcus aureus* strains showed sensitivity reactions with nine (ciprofloxacin, gentamicin, chloramphenicol, tetracycline, refampicin, augmentin, penicillin, nitrofurantoin, and sparfloxacin) out of the twelve antibiotics used. SA1 recorded a Mean±S.E (standard error) zone of inhibition of 9.5±2.82mm with ciprofloxacin, gentamcin, augmentin, chloramphenicol, tetracycline, refapicin and sparfloxacin. The highest and least Mean±SE zones of inhibition were recorded by SA5 and SA7 which was 14.3±2.93 and 5.75±2.38 respectively. *S. aureus* strains SA2, SA3, SA4 and SA6 recorded Mean±S.E zones of inhibition of 7.8±2.34mm, 7.5±2.92mm, 8.10±3.03mm and 9.0 ±3.48mm respectively. All seven *Staphylococcus aureus* strains did not show any visible reaction with amoxicillin, ampicillin and nalidixic acid. All but SA1, SA5 and SA7 strains were resistant to chloramphenicol. Also all but SA5 and SA7 were resistant to penicillin. The highest (26.3±1.54mm) and lowest (3.29±2.25mm) zones of inhibition recorded by all the strains were reactions with ciprofloxacin and penicillin respectively. 16.9±3.57mm, 8.0±2.39mm, 4.7±2.36, 14±1.20, 18.4±4.09mm, 9.6±2.66mm, 5.1±2.54mm zones of inhibition were sensitivity reactions recorded to gentamicin, augmentin, chloramphenicol, tetracycline, rifampicin, nalidixic acid and sparfloxacin respectively.

Table 1: Sensitivity Profile of *Staphylococcus aureus* strains before Treatment with Acridine orange after incubation at 37^{0}C for 24 hours

	Zones of inhibition (mm) around antibiotic discs												Mean±S.E
Isolate	CIP	GEN	AUG	CHL	AMX	AMP	TET	REF	PEN	NAL	NT	SP	
SA1	30.0	18.0	10.0	R	R	R	15.0	15.0	R	R	R	16.0	9.5 ±2.82
SA2	19.0	10.0	10.0	R	R	R	10.0	25.0	R	R	10	R	7.8±2.34
SA3	25.0	15.0	R	R	R	R	10.0	25.0	R	R	15.0	R	7.5±2.92
SA4	29.0	25.0	8.0	R	R	R	15.0	9.0	R	R	10.0	R	8.1±3.03
SA5	29.0	25.0	18.0	0	R	R	17.0	25.0	15.0	R	17.0	10.0	14.3±2.93
SA6	25.0	25.0	10.0	R	R	R	18.0	30.0	R	R	R	R	9.0±3.48
SA7	23.0	R	R	8.0	R	R	13.0	R	8.0	R	15.0	R	5.75±2.38
Mean±S.E	26.3±1.54	16.9±3.57	8.0±2.39	4.7±2.36	0	0	14.0±1.20	18.4±4.09	3.29±2.25	0	9.6±2.66	5.1±2.54	

KEY: AMP = ampicillin, CIP = ciprofloxacin, GN= gentamicin, CHL=chloramphenicol AMX= amoxicillin, AUG= augmentin, TET=tetracycline, RE= Rifampicin, PN= penicillin, NAL= nalidixic acid, NT= nitrofurantion, SP = sparfloxacin, R = resistance

The susceptibility of *S. aureus* to various antibiotics is presented in Table 2. The results showed high resistance to nalidixic acid, ampicillin and amoxicillin with a percentage resistance of 100%. The isolates were 100% susceptible to ciprofloxacin and tetracycline and 86.7% susceptible to gentamicin and rifampicin. Percentage susceptibility to gentamicin and nitrofurantoin was 71.4%.

Table 3 shows *Staphylococcus aureus* isolates SA1, SA2, SA3, SA4, SA5, SA6, and SA7 from high vaginal and urethral swabs and their sensitivity reactions to ciprofloxacin, gentamicin, augmentin, chloramphenicol, ampicillin, tetracycline, amoxicillin, rifampicin, penicillin, nalidixic acid, nitrofurantoin and sparfloxacin after treatment with acridine orange. The seven *S. aureus* strains showed sensitivity reactions with the twelve antibiotics tested. The highest Mean±S.E (standard error) zone of inhibition recorded was 26.60±0.61mm for SA5 while the least Mean±S.E zone of inhibition recorded was 13.33±2.92mm for SA4. The isolates showed resistance only to ampicillin, chloramphenicol, penicillin and nalidixic acid and only SA1 and SA4 retained their resistant gene.

In Table 4, the percentage loss of resistance markers due to curing effect of acridine orange is shown. Loss of 50% and above of resistance markers was recorded for *S. aureus* strain SA1with 53.3% , 50% and 56.5% loss of resistance to tetracycline, rifampicin and sparfloxacin after treatment with acridine orange. Strain SA2 showed 57.9%, 66.7%, 93.3% and 200% loss of resistance markers to ciprofloxacin, gentamicin, nitrofurantoin and sparfloxacin. Resistance markers present in all seven strains to ampicillin, amoxicillin and nitrofurantoin, remained unchanged after treatment with acridine orange although appreciable zones of inhibition were recorded.

TABLE 2: PERCENTAGE OCCURRENCE OF DRUG RESISTANCE OF *Staphylococcus aureus* STRAINS

Antibiotics Tested	Percentage sensitivity	Percentage resistance
Ciprofloxacin	7 (100)	0(0)
Gentamicin	6(85.7)	1(14.3)
Augmentin	5(71.4)	2(28.6)
Chloramphenicol	3(42.9)	4(57.1)
Amoxicillin	0(0)	7(100)
Ampicillin	0(0)	7(100)
Tetracycline	7(100)	0(0)
Rifampicin	6(85.7)	1(14.3)
Penicillin	2(28.6)	5(71.4)
Nalidixic acid	0(0)	7(100)
Nitrofurantion	5(71.4)	2(28.6)
Sparfloxacin	2(28.6)	5(71.4)

Table 3: Sensitivity Profile of *Staphylococcus aureus* strains after Treatment with acridine orange and incubation at 37^{0}C for 24 hours

	Zones of inhibition (mm) around antibiotic discs												Mean±S.E
Isolate	AMP	CIP	GEN	AUG	CHL	AMX	TET	REF	PEN	NAL	NT	SP	
SA1	10.0	30.0	25.0	10.0	10.0	10.0	25.0	10.0	R	R	10.0	25.0	12.8±3.10
SA2	25.0	30.0	25.0	25.0	28.0	25.0	25.0	25.0	25.0	25.0	25.0	30.0	26.1±0.60
SA3	25.0	29.0	25.0	25.0	28.0	25.0	25.0	25.0	26.0	25.0	29.0	20.0	25.6±0.70
SA4	R	30.0	25.0	10.0	10.0	8.0	25.0	10.0	R	8.0	10.0	25.0	13.3±2.92
SA5	30.0	30.0	25.0	25.0	25.0	29.0	29.0	25.0	25.0	27.0	25.0	25.0	26.6±0.61
SA6	25.0	29.0	20.0	25.0	20.0	25.0	20.0	30.0	25.0	25.0	25.0	25.0	24.5±0.93
SA7	20.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	20.0	25.0	25.0	20.0	23.6±0.7
Mean±S.E	19.3±4.00	28.4±0.92	24.3±0.72	20.7±2.80	19.4±4.00	21.0±3.20	24.6±1.02	21.4±3.03	17.3±4.52	19.3±4.10	21.3±2.98	24.3±1.32	

KEY: AMP = ampicillin, CIP = ciprofloxacin, GN= gentamicin, CHL=chloramphenicol AMX= amoxicillin, AUG=augmentin, TET=tetracycline, REF= rifampicin, PEN= penicillin, NAL= nalidixic acid, NT= nitrofurantion, SP = sparfloxacin, R = resistance

Table 4: Percentage sensitivity enhancement of *Staphylococcus aureus* after treatment with Acridine orange and incubation at 37^{0}C

Zones of Inhibition (in mm) Around Antibiotic Discs Before and After treatment with acridine orange

ISOLATES	CIP	GEN	AUG	CHL	AMX	AMP	TET	RE F	PEN	NAL	NT	SP
SA1												
Before	30.0	18.0	10.0	10.0	0.0	0.0	15.0	10.0	0.00	0.0	0.00	16.0
After	30.0	25.0	10.0	0.00	10.0	10.0	23.0	15.0	25.0	0.0	10.0	25.0
	(0.0%)	(38.9%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(53.3%)	(50.0%)	(0.0%)	(0.0%)	(0.0%)	(56.5%)
SA2												
Before	19.0	10.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	10.0	10.0
After	30.0	25.0	25.0	28.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	30.0
	57.9%	66.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	93.3%	200.0%
SA3												
Before	25.0	15.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	15	0.0
After	29.0	25.0	25.0	28.0	25.0	25.0	25.0	25.0	26.0	25.0	29.0	20.0
	16.0%	66.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	93.3%	0.0%
SA4												
Before	29.0	25.0	8.0	0.0	0.0	0.0	9.0	0.0	0.0	0.0	10.0	0.0
After	30.0	25.0	10.0	10.0	8.0	15.0	25.0	10.0	0.0	0.0	10.0	25.0
	3.4%	0.0%	25.0%	0.0%	0.0%	0.0%	177.0%	0.0%	0.0%	0.0%	0.0%	0.0%
SA5												
Before	29.0	25.0	18.0	15.0	0.0	0.0	17.0	25.0	15.0	0.0	17	10.0
After	30.0	25.0	25.0	25.0	29.0	30.0	29.0	25.0	25.0	27.0	25.0	25.0
	3.4%	0.0%	70.0%	66.7%	0.0	0.0	70.6%	0.0	66.7%	0.0	47.1%	150.0%
SA6												
Before	25.0	0.0	0.0	8.0	0.0	0.0	13.0	0.0	8.0	0.0	15.0	0.0
After	29.0	20.0	25.0	20.0	25.0	25.0	20.0	30.0	25.0	25.0	25.0	25.0
	16.0%	0.0%	0.0%	150.0%	0.0%	0.0%	53.8%	0.0%	212.0%	0.0%	66.7%	0.0%
SA7												
Before	23.0	0.0	0.0	8.0	0.0	0.0	13.0	0.0	8.0	0.0	15.0	0.0
After	25.0	25.0	25.0	25.0	25.0	20.0	25.0	25.0	20.0	25.0	25.0	20.0
	(8.7%)	(0.0%)	(0.0%)	(212.5%)	(0.0%)	(0.0%)	(92.3%)	(0.0%)	(150.0%)	(0.0%)	(66.7%)	(0.0%)

DISCUSSION

In this study, acridine orange modified according to non-toxic laboratory concentrations of ethidium bromide reported by Wurmb-Schwark *et al*. (2006) was used to treat and reduce resistance markers present in multidrug resistant *Staphylococcus aureus* strains isolated from urethral swab and high vaginal swab. The antibiotic susceptibility patterns of all seven *Staphylococcus aureus* strains before acridine orange treatment in this study showed that the highest to the least sensitive drugs were ciprofloxacin, tetracycline, gentamicin, rifampicin, augmentin, nitrofurantoin, chloramphenicol, penicillin, sparfloxacin, ampicillin, amoxicillin and

nalidixic acid. *S. aureus* strains were all completely resistant to ampicillin, amoxicillin and nalidixic acid (Table 1). The sensitivity of all the *Staphylococcus aureus* strains to the first four drugs is encouraging as these drugs are not expensive. Hence, choices can be made between ciprofloxacin, tetracycline, gentamicin and rifampicin, or a combination of any two in the treatment of infections due to multidrug resistant *Staph. aureus*. The total resistance recorded against ampicillin and amoxicillin is worrisome because these drugs are used routinely to treat a myriad of human diseases. This same worry with particular reference to amoxicillin was expressed by Otajevwo and Momoh, (2013) were 100% resistance to amoxicillin was recorded in their report on resistance marker loss of multi-drug resistant *Staphylococcus aureus* strains after treatment with dilutions of acridine orange. It was not clear as to whether the site from where the pathogens were isolated had any direct or indirect effect on the sensitivity patterns recorded.

A pathogen is multidrug resistant (MDR) when it is resistant to three or more antibiotics at any given time (John and Sentry, 2004). This finding re-establishes the multidrug resistant nature of *Staphylococcus aureus* strains irrespective of the source or site from where they are isolated. Indeed, many authors have profusely reported occurrence of multidrug resistant *S. aureus* in their studies (Daniyan and Sani, 2011; Otajevwo and Momoh, 2013). The high prevalence of multiple antibiotic resistant *Staph. aureus* strains in this study is a possible suggestion that very large population of *S. aureus* organisms has been exposed to several antibiotics which is consistent with the report of Otajevwo and Momoh, (2013). This result of high resistance was also reported by Daniyan and Sani, (2011) were 89.29% resistance was recorded when they investigated the antibiotics susceptibility patterns of *staphylococcus aureus isolated* from some clinical samples in a secondary health care.

Acridine orange was used to treat and cure the seven *Staphylococcus aureus* strains with the intention of reducing their resistance markers significantly or eliminating them completely. The loss of 50%-100% of resistance markers after treatment with acridine orange was used as the basis of establishing the curing effects. The use of 50% and above loss in resistance markers as a criterion to determine extent of plasmid curing was consistent with the report of Akortha *et al*., (2011). Stanier *et al*. (1984) reported that the elimination of plasmids by dyes and other agents reflects the ability of such agents to inhibit plasmid replication at a concentration that does not affect the chromosome.

CONCLUSION

Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates. In this study,

all seven *Staphylococcus aureus* isolates were sensitive to 9(75%) of the antibiotics used which included ciprofloxacin, gentamicin, chloramphenicol, tetracycline, refampicin, augmentin, penicillin, nitrofurantoin, and sparfloxacin. The highest and least mean± standard error zones of inhibition which were 14.3±2.93mm and 5.75±2.38mm were recorded by MDR *Staphylococcus aureus* strains SA5 and SA7 respectively. *S. aureus* strains SA4 and SA5 were isolated from high vagina swab and urethral swab respectively. The highest Mean±S.E zone of inhibition recorded by the seven strains to the various antibiotics was 26.3±1.54, 18.4±4.09 and 16.9±3.57 for ciprofloxacin, refampicin and gentamicin respectively. Thus this finding suggests the use of these drugs in the treatment of diseases caused by multiple drug resistant *Staphylococcus aureus* pathogens. Futhermore, isolates should always be subjected to antimicrobial susceptibility testing before administering of drugs. This measure can reduce the burden of antimicrobial resistance and prevent a public health problem.

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