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Resistance marker loss of multi-drug resistant (MDR) *staphylococcus aureus* strains after treatment with dilutions of acridine orange

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This investigation was aimed at studying resistance marker loss of multidrug resistant (MDR) *Staphylococcus aureus* strains after treatment with sub-clinical dilutions of acridine orange. Seven (7) pure axenic strains of *Staph aureus* coded SA_1-SA_7 isolated from seven infected human sources which included seminal fluid, ear swab, midstream urine, wound swab, urethral swab, high vaginal swab and endocervical swab respectively, were obtained from the Medical Microbiology laboratory of the Delta State University Teaching Hospital, Oghara, Nigeria and stocked on sterile Nutrient agar slants. Colonies from resulting stocked cultures were re-confirmed by subculturing them on sterile Mannitol Salt agar plates and incubated at 37^oC for 24hours. Gram staining, catalase and coagulase tests were carried out on resulting colonies. Antibiotic sensitivity test by agar disc diffusion method was done on confirmed strains on sterile Mueller Hinton agar plates before and after treatment with acridine orange (AO). *Staph aureus* strains that showed ≤50.0% reduction in resistance marker after treatment with 0.35, 0.55, 0.75 and 0.95ug/ml dilutions of AO were noted. Minimum inhibitory concentration (MIC) assay was done using gentamicin on SA_1 strain with all four dilutions of AO. All seven strains showed resistance against augmentin, amoxicillin and cloxacillin. The highest (19.9± 2.3mm) and lowest (6.6 ± 7.3mm) zones of inhibition recorded by all the strains were reactions to chloramphenicol and cotrimoxazole respectively. Strains SA_4, SA_2 and SA_7 recorded 17.0±4.9mm, 14.4±3.3mm, 14.0±5.9mm and 7.3±4.6mm mean zones of inhibition to gentamicin, erythromycin, chloramphenicol and tetracycline respectively. Strains SA_2, SA_4 and SA_7 were each resistant to three of the total antibiotics used. Strains SA_6, SA_1, SA_3 and SA_5 were resistant to four, five, five and six of the antibiotics used respectively. Mean loss of 50.0% or more of resistance marker (RM) was recorded after treatment with 0.35ug/ml (60.0%), 0.95ug/ml (52.7%) and 0.75ug/ml (52.2%) to erythromycin. There was zero loss of RM to gentamicin, chloramphenicol and tetracycline after treatment with 0.55ug/ml, 0.35ug/ml and 0.75ug/ml AO respectively. Acridine orange dilutions of 0.95 and 0.35ug/ml produced an eight-fold (1.25ug) and four-fold (2.5ug) reduction respectively in the minimum inhibitory concentration of gentamicin. The implications of these findings are discussed.

Keywords: Loss, resistance marker, multidrug resistant Staph. aureus strains, treatment, acridine orange, dilutions.

INTRODUCTION

Antimicrobial resistance is on the rise in Europe and 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; Mustapha *et al.*, 2009).This selective pressure is the result of indiscriminate use of antibiotics, complex

socio-economic behavioral antecedents, dissemination of drug resistant pathogens in human medicine (Okeke *et al.*, 1999) 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 resistance genes among pathogens which are often felicitated by the localization of these genes on plasmids particularly those associated with integrons and transposons (Tenover, 2006).

Multidrug resistance is now common in familiar pathogens such as *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* to mention a few (Nabeela *et al.,* 2004).

The last decade witnessed the emergence of *Staph. aureus* as a deadly superbug. The enormous genetic plasticity of the organism assists it to endlessly evolve resistance mechanisms against existing anti-microbial 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 90% of *Staph. 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 (Nichols, 1999). While vancomycin is often regarded as the last line of defense against nosocomial and community-based *Staph.aureus* infections (Bhalakia, 2008), resistance has been reported and there is a major concern that total antibiotic resistant strains may emerge in the immediate future (Diekema *et al.*, 2001).

Soon after the use of penicillin, *Staphylococcus aureus* was found to produce penicillinase (beta-lactamase). To overcome this situation, the antibiotic-methicillin was used to replace penicillin and *Staph. 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 resulting bacterial organism then becomes sensitive to the selective agent and it was initially thought that this phenomenon would proffer solution in controlling the development of antibiotic 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 (effectiveness) of antibiotics in situ. There is dearth of literature on antibiotic sensitivity improvement using sub-lethal dilutions of chemical agents such as acridine orange. Hence, the aim of this work is to study the resistance marker loss of multidrug resistant *Staphylococcus aureus* strains after treatment with sub-clinical dilutions of acridine orange with the following objectives: (1) Determine sensitivity profile of *Staphylococcus aureus* strains before treatment with dilutions of acridine orange after incubation at 37°C for 24 hours. (2) Determine distribution of multidrug resistance markers among the selected pathogenic strains of *staphylococcus aureus*. (3) Determine *Staph. aureus* strains that showed ≤50.0% loss of their resistance markers after treatment with 0.35µg/ml dilution of acridine orange and incubation at 37°C for 24hrs. (4) Determine *Staph. aureus* strains that showed ≤50.0% loss of their resistance markers after 0.55 µg/ml acridine orange dilution treatment. (5) Determine *Staph. aureus* strains that showed ≤50.0% loss of their markers after 0.75 µg/ml acridine orange dilution treatment. (6) Determine *Staph. aureus* strains that showed ≤50.0% loss of their markers after 0.95µg/ml acridine orange dilution treatment. (7) Determine the distribution of ≤50.0% loss in resistance markers among multidrug resistant *Staph. aureus* strains after treatments with 0.35, 0.55, 0.75 and 0.97µg/ml dilutions of acridine orange. (8) Acridine orange dilutions' effect(s) on the minimum inhibitory concentration (MIC) of gentamicin (an aminoglycoside) on an MDR *Staph. aureus* strain isolated from seminal fluid after 24hrs incubation at 37°C.

MATERIALS AND METHODS

Sampling

Seven pure (axenic) strains of *Staphylococcus aureus* coded SA_1 – SA_7 were obtained from the Medical Microbiology department of Delta State University Teaching Hospital, Oghara, Nigeria. Bacterial pathogens which were isolated from seminal fluid, ear swab, midstream urine, wound swab, urethral swab, high vaginal swab and endocervical swab respectively, were inoculated aseptically on sterile nutrient agar slants and

incubated at 37°C for 24hours. All strains were appropriately labeled.

Processing of Samples

Colonies from resulting stocked cultures were re-confirmed by sub-culturing them on sterile Mannitol Salt agar plates and incubated for 24hours at 37°C. Colonies were then identified by standard methods (Cowan and Steel, 1993). Bright yellow, smooth gram positive *cocci* in clusters, catalase positive, coagulase positive colonies which were confirmatory of *Staphylococcus aureus* were then stocked on sterile Nutrient agar slants and kept at 4°c in the refrigerator for further use. The set up was appropriately labeled. All isolated bacterial pathogens were subjected to antibiotic sensitivity testing before treatment with subclinical dilutions of acridine orange.

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing on each of the seven pure strains of *Staph.aureus* was carried out using the agar disc diffusion method on sterile Muller-Hinton agar (MHA) plates (Bauer *et al.*, 1996). A loopful of each colony was picked aseptically using a flamed wire loop and placed in the center of sterile MHA 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 and cooled and used to pick an antibiotic multidisc (Abitek, Liverpool) containing augmentin (30ug), amoxicillin (25ug), erythromycin (5ug), tetracycline (10ug), cloxacillin (5ug), gentamycin (10ug), cotimoxazole (25ug), and chloramphenicol (30ug). Discs were placed at least 22.0mm from each other and 14.0mm from the edge of the plate (Ochei and Kolhatkar, 2008). Antibiotic discs were selected on the basis of their clinical importance and efficacy on *Staph.aureus.* Plates were allowed to stand for 10minutes before incubation (Mbata, 2007). The plates were incubated at 37°c for 24hours. A reference control strain of *Staph.aureus* NCTC was inoculated in the same way on another plate with the same antibiotic discs and incubated at the same temperature and time. At the end of incubation, the diameters of the zones of inhibition from one edge to the opposite edge were measured to the nearest millimeter using a transparent ruler (Byron *et al.*, 2003). Strains that showed resistance against three antibiotics and above were termed multiple drug resistant (MDR) strains (Jan *et al.*, 2002) and were noted and used further.

Preparation of Sub-clinical Laboratory Dilutions of Acridine Orange

For treatment of MDR pathogens, sub-clinical acridine orange concentrations of 0.35, 0.55, 0.75 and 0.95µg/ml

were used. These concentrations (dilutions) were chosen in line with non-toxic laboratory concentrations of 0.25-1.0µg/ml prescribed by Wurmb-Schwark *et al.* (2006) for ethidium bromide which have been reported to posseess curing potentials (Otajevwo, 2012). According to Wurmb-Schwark*et al.* (2006), 0.25-1.0µg/ml dilutions of ethidium bromide are safe for laboratory use as they are below toxicity levels. Hence, acridine orange dilutions of 0.35, 0.55, 0.75 and 0.95µg/ml were prepared using RV/O where stock or original concentration of acridine orange used was 1.0mg/ml or 1000µg/ml. To obtain 0.35µg/ml dilution therefore, 0.14ml of stock reagent was added to sterile 9.86ml of Mueller Hinton broth. To obtain 0.55µg/ml acridine orange dilution, 0.11ml stock acridine orange solution was added to sterile 19.78ml Mueller-Hinton broth. Also to obtain 0.75µg/ml dilution, 0.30ml stock solution was added to sterile 19.70ml Mueller-Hinton solution and 0.38ml stock solution of acridine orange was added to sterile 19.61ml of Mueller-Hinton broth to obtain 0.95µg/ml dilution. All dilutions were effected in sterile universal bottles containing sterile 20.0ml of Mueller-Hinton broth each.

Growing Broth Culture of MDR Pathogens

A colony of each MDR pathogen strain was aseptically picked from its slant stock culture using flamed and cooled wire loop and inoculated into sterile 10ml Mueller-Hinton broth. Inoculated broths were incubated at 37°C for 18 hours. The resulting turbid broth culture was then diluted according to a modified method of Shirtliff *et al.* (2006). Using a sterile pipette, 0.1ml of broth culture was mixed with 99.9ml (1:200 dilution) of sterile Mueller-Hinton broth. This was properly mixed and was used as working inoculum and should contain 10^5-10^6 organisms if used within 30minutes (Ochei and Kolhatkar, 2008).

Treatment of MDR Pathogenic Strains with Prepared Acridine Orange Dilutions

The treatment of MDR *Staph aureus* pathogenic strains with the prepared dilutions of 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. The various prepared dilutions (one at a time) of acridine orange were then added in 0.5ml volume. The set up was properly mixed and labeled. The set up for each dilution was then poured on top of sterile hardened or set 2% Nutrient agar plates and left to set. 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°C for 24hours. Measurement of diameters of zones of inhibition were taken and recorded (NCCLS, 2000).

Table 1. Sensitivity Profile of *Staphylococcus aureus* strains before Treatment with subclinical dilutions of Acridine orange after incubation at 37°C for 24 hours

Strain codes	Zones of inhibition (mm) around Antibiotic Discs								Mean±S.E
	AUG	ERY	GEN	CHL	TET	COT	AMX	CXC	
SA_1	R	10.0	15.0	22.0	R	R	R	R	9.4±5.04
SA_2	R	12.0	16.0	21.0	9.0	14.0	R	R	14.4±3.29
SA_3	R	5.0	15.0	20.0	R	R	R	R	8.0±5.19
SA_4	R	9.0	20.0	20.0	11.0	25.0	R	R	17.0±4.92
SA_5	R	R	12.0	17.0	R	R	R	R	5.8±4.67
SA_6	R	11.0	13.0	16.0	17.0	R	R	R	11.4±2.71
SA_7	R	5.0	21.0	23.0	14.0	7.0	R	R	14.0±5.88
Mean ±S.E	**0.0**	**7.43±2.69**	**16.0±3.01**	**19.9±2.28**	**7.3±4.56**	**6.57±7.25**	**0.0**	**0.0**	

AUG=augmentin, ERY=erythromycin, GEN=gentamycin, CHL=chloramphenicol, TET=tetracycline, COT=cotrimoxazole, AMX=amoxicillin, CXC=cloxacillin.

Determination of Minimum Inhibitory Concentration (MIC) of Gentamicin after Acridine Orange Treatment

Serial doubling dilutions of gentamycin, one the antibiotics to which a *Staph aureus* strain (isolated from seminal fluid) recorded more than 50.0% resistance marker loss was carried out. The antibiotic being used for this assay was sterilized by filtration before use. Sterile test tubes, numbering 13 were set up on a test tube rack and labeled 1-13. Using a sterile pipette, 1ml of diluted broth was dispensed into tubes 2-10, 11 and 13. Into tube 12, 2.0ml of diluted broth culture was pipetted. Tube 11 was the inoculum control, tube 12 was the broth control and 13 was the drug control. Into tubes 1, 2 and 13, 1ml of the working antibiotic solution was pipetted. Serial doubling dilutions of antibiotic solution were separately prepared in Nutrient broth to get reducing concentrations of the antibiotic using 100-0.4µg/ml as a standard for MIC assay (Ochei and Kolhatkar, 2008). All tubes were incubated at 37°C for 18hours. Turbidity (cloudiness) in the growth medium indicated growth. Tube 11 should show turbidity and tubes 12 and 13 should show no growth. The lowest concentration showing no growth was the MIC of the antimicrobial agent as effective against *Staph. aureus* strains. All MIC results before and after treatment with dilutions of acridine orange were recorded accordingly.

RESULTS

Table 1 shows *Staphylococcus aureus* strains SA_1, SA_2, SA_3, SA_4, SA_5, SA_6, and SA_7 isolated from seminal fluid, ear swab, mid-stream urine, wound swab, urethral swab, high vaginal swab and endocervical swab respectively and their sensitivity reactions to augmentin, erythromycin, gentamycin, chloramphenicol, tetracycline, cotrimoxazole (septrin), amoxicillin and cloxacillin.
Before treatment with dilutions of acridine orange, the seven *Staph.aureus* strains showed sensitivity reactions with five (erythromycin, gentamycin, chloramphenicol, tetracycline and cotrimoxazole) out of the eight antibiotics used. SA_1 recorded a mean ±S.E (standard error) zone of inhibition of 9.4±5.04mm with erythromycin, gentamycin and chloramphenicol. The highest and least mean ±SE zones oh inhibition recorded by SA_4 and SA_5 were 17.0±4.92 and 5.8±4.67 respectively.
Staph.aureus strains SA_2, SA_4 and SA_7 recorded mean±S.E zones of inhibition of 14.4±3.29mm, 17.0±4.92mm and 14.0±5.88mm respectively with erythromycin, gentamycin, chloramphenicol, tetracycline and cotrimoxazole. Strains SA_3 and SA_5 recorded 8.0±5.19mm and 5.8±4.67mm zones of inhibition respectively with gentamycin and chloramphenicol as well as erythromycin for SA_3. Apart from cotrimoxazole, SA_6 strain recorded 11.4±2.71mm inhibition zone with the remaining four antibiotics.
All the seven *Staph.aureus* strains did not show any visible reaction with augmentin, amoxicillin and cloxacillin. All but SA_2, SA_4 and SA_7 strains were resistant to cotrimoxazole (septrin). The highest (19.86±2.28mm) and lowest (6.57±7.25mm) zones of inhibition recorded by all the strains were reactions with chloramphenicol and cotrimoxazole respectively. The next highest zone of inhibition by all strains (16.0±3.01mm) was sensitivity reaction with gentamycin. Similarly, the next lowest (7.29±4.56mm) was reaction with tetracycline.
The occurrence of multidrug resistant *Staph.aureus* strains as shown by antibiotic susceptibility reactions is shown in Table 2.
Strains SA_2 (isolated from ear swab), SA_4 (wound swab) and SA_7 (endocervical swab) were resistant to three antibiotics which included augmentin, amoxicillin and cloxacillin. Augmentin, amoxicillin, cloxacillin and cotrimoxazole (4 antibiotics) were resisted by strain SA_6 (isolated from high vaginal swab). Five antibiotics (augmentin, amoxicillin, tetracycline, cloxacillin and cotrimoxazole) were resisted each,by SA_1 (seminal fluid) and SA_3 (midstream urine). Lastly, SA_5 (urethral swab) strain was resistant to augmentin, amoxicillin,

Table 2. Occurrence of multidrug resistance among MDR pathogenic strains of *Staph.aureus.*

Strain Code	3 Drugs	4 Drugs	5 Drugs	<5 Drugs	Drugs Resisted
SA$_2$	+	-	-	-	Augmentin, Amoxil, Cloxacillin
SA$_4$	+	-	-	-	Augmentin, Amoxil, Cloxacillin
SA$_7$	+	-	-	-	Augmentin, Amoxil, Cloxacillin
SA$_6$	-	+	-	-	Augmentin, Amoxil, Cloxacillin, Cotrimoxazole
SA$_1$	-	-	+	-	Augmentin, Amoxil, Tetracycline, Cloxacillin, Cotrimoxazole
SA$_3$	-	-	+	-	Augmentin, Amoxil, Tetracycline, Cloxacillin, Cotrimoxazole
SA$_5$	-	-	-	+	Augmentin, Amoxil, Erythromycin, Tetracycline, Cloxacillin, Cotrimoxazole

Table 3. *Staph.aureus* strains that showed ≤50.0% sensitivity enhancement after treatment with 0.35µg/ml (non-toxic) dilutions of Acridine orange and incubation at 37^0c for 24hrs.

MDR Strains		Zones of Inhibition (in mm) Around Antibiotic Discs before and After treatment with 0.35ug/ml dilution of Acridine orange						
		AUG	ERY	GEN	CHL	TET	COT	AMX
SA$_1$	Before	0.0	10.0	15.0	22.0	0.0	0.0	0.0
	After	23.0	16.0	20.0	24.0	19.0	14.0	19.0
		(0.0%)	**(60.0%)**	(33.3%)	(9.9%)	(0.0%)	(0.0%)	(0.0%)
SA$_2$	Before	0.0	12.0	16.0	21.0	9.0	0.0	0.0
	After	0.0	16.0	21.0	25.0	19.0	0.0	20.0
		(0.0%)	(33.3%)	(31.3%)	(19.1%)	**(111.1%)**	(0.0%)	(0.0%)
SA$_3$	Before	0.0	5.0	15.0	20.0	0.0	0.0	0.0
	After	4.0	12.0	20.0	22.0	15.0	15.0	10.0
		(0.0%)	**(140.0%)**	(33.3%)	(10.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_4$	Before	0.0	9.0	20.0	20.0	11.0	25.0	0.0
	After	0.0	18.0	24.0	24.0	19.0	27.0	14.0
		(0.0%)	**(100.0%)**	(20.0%)	(20.0%)	**(72.7%)**	(17.3%)	(0.0%)
SA$_5$	Before	0.0	0.0	12.0	17.0	0.0	0.0	0.0
	After	0.0	12.0	26.0	21.0	0.0	0.0	0.0
		(0.0%)	(0.0%)	**(116.6%)**	(23.5%)	(0.0%)	(0.0%)	(0.0%)
SA$_6$	Before	0.0	11.0	13.0	16.0	17.0	0.0	0.0
	After	0.0	11.0	21.0	18.0	21.0	0.0	18.0
		(0.0%)	(0.0%)	**(61.5%)**	(12.5%)	**(116.7%)**	(0.0%)	(0.0%)
SA$_7$	Before	0.0	5.0	21.0	23.0	14.0	7.0	0.0
	After	0.0	11.0	26.0	27.0	19.0	7.0	0.0
		(0.0%)	**(120.0%)**	(23.8%)	(17.3%)	(35.7%)	(0.0%)	(0.0%)

≤50.0% Resistance Marker Losses are highlighted.

erythromycin, tetracycline, cloxacillin and cotrimoxazole (6 drugs).

In Table 3, the percentage loss of resistance markers due to 0.35µg/ml curing effect of acridine orange is shown. Loss of 50% and above of resistance markers was recorded for *Staph.aureus* strain SA$_1$ with 60% loss of resistance to erythromycin after treatment with 0.35µg/ml acridine orange. Strain SA$_2$ showed 111.1% loss of resistance markers to tetracycline after the same treatment. Strains SA$_3$, SA$_4$ and SA$_7$ recorded loss of resistance markers of 140%, 100% and 120% respectively to erythromycin which was far higher compared to 60% loss recorded by SA$_1$ to erythromycin.

Strains SA$_4$ and SA$_6$ recorded 72.7% and 116.7% resistance marker loss respectively to tetracycline. There was also 61.5% resistance loss to gentamycin by SA$_6$. Resistance markers present in all seven strains to augmentin, amoxicillin and cotrimoxazole, remained unchanged after 0.35µg/ml acridine treatment although appreciable zones of inhibition were recorded after treatment on SA$_1$ (with augmentin and cotrimoxazole), SA$_3$ (with augmentin and cotrimoxazole), SA$_4$ (with cotrimoxazole) and SA$_7$ (with cotrimoxazole).

Table 4 shows percentage loss of resistance markers due to 0.55µg/ml curing effect of acridine orange loss of 50% and above resistance markers after treatment is

Table 4. *Staph.aureus* strains that showed ≤50.0% sensitivity enhancement after treatment with 0.55µg/ml dilutions of Acridine orange and incubation at 37^0c for 24hrs.

MDR Strains		Zones of Inhibition (in mm) Around Antibiotic Discs before and After treatment with 0.55ug/ml dilution of Acridine orange						
		AUG	ERY	GEN	CHL	TET	COT	AMX
SA$_1$	Before	0.0	10.0	15.0	22.0	0.0	0.0	0.0
	After	0.0	20.0	16.0	25.0	8.0	0.0	20.0
		(0.0%)	**(100.0%)**	(6.7%)	(13.6%)	(0.0%)	(0.0%)	(0.0%)
SA$_2$	Before	0.0	12.0	16.0	21.0	9.0	14.0	0.0
	After	0.0	13.0	18.0	26.0	15.0	17.0	0.0
		(0.0%)	(8.3%)	(12.5%)	(23.8%)	**(66.7%)**	(21.4%)	(0.0%)
SA$_3$	Before	0.0	5.0	15.0	20.0	0.0	0.0	0.0
	After	0.0	6.0	19.0	23.0	0.0	0.0	25.0
		(0.0%)	(20.0%)	(26.7%)	(15.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_4$	Before	0.0	9.0	20.0	20.0	11.0	25.0	0.0
	After	0.0	13.0	20.0	22.0	11.0	26.0	0.0
		(0.0%)	(44.4%)	(0.0%)	(10.0%)	(0.0%)	(4.0%)	(0.0%)
SA$_5$	Before	0.0	0.0	12.0	17.0	0.0	0.0	0.0
	After	0.0	0.0	15.0	20.0	10.0	0.0	18.0
		(0.0%)	(0.0%)	(25.0%)	(17.6%)	(0.0%)	(0.0%)	(0.0%)
SA$_6$	Before	0.0	11.0	13.0	16.0	17.0	0.0	0.0
	After	0.0	16.0	18.0	25.0	18.0	0.0	0.0
		(0.0%)	(45.5%)	(38.5%)	**(56.3%)**	(5.9%)	(0.0%)	(0.0%)
SA$_7$	Before	0.0	5.0	21.0	20.0	14.0	7.0	0.0
	After	0.0	7.0	25.0	23.0	16.0	8.0	11.0
		(0.0%)	(40.0%)	(19.0%)	(15.0%)	(14.3%)	(14.3%)	(0.0%)

≤50.0% Resistance Marker Losses are highlighted.

shown by *Staph. aureus* strains SA$_1$, (100% loss to erythromycin), SA$_2$ (66.7% loss to tetracycline) and SA$_6$ (56.3% loss to chloramphenicol). Resistance markers present in all seven strains of augmentin and amoxicillin remained unchanged after 0.55µg/ml acridine orange treatment. There was improved reduction in resistance markers to cotrimoxazole.

Data on 50% and above reduction in resistance marker in *Staph.aureus* strains after treatment with 0.75µg/ml acridine orange are shown in Table 5. Strains SA$_1$, SA$_2$, SA$_3$ and SA$_4$ recorded 140%, 50%, 120% and 55.6% reduction in resistance markers to erythromycin. Strains SA$_2$ and SA$_5$ showed 56.3% and 75.0% loss of resistance markers to gentamycin after treatment with 0.75µg/ml acridine orange. Loss of resistance markers to chloramphenicol by SA$_3$, SA$_5$ and SA$_7$ was 100.0%, 70.0% and 64.3% respectively. Resistance markers present in the seven *Staph. aureus* strains to augmentin and amoxicillin remained unaltered after treatment with 0.75µg/ml acridine orange. Slight improvement in zones of inhibition was recorded for SA$_2$ to tetracycline and cotrimoxazole after treatment. Similarly, SA$_4$ recorded appreciable improvement in zones of inhibition tocotrimoxazole and amoxicillin while SA$_6$ recorded improvement in zones of inhibition to tetracycline and amoxicillin. Strain SA$_7$ also recorded enhanced improvement in zones of inhibition to tetracycline, cotrimoxazole and amoxicillin after same treatment.

The effect of 0.95µg/ml acridine orange treatment of *Staph.aureus* strains on the selected antibiotics is shown in

Table 6. Strains SA$_1$ (semen fluid), SA$_4$ (wound swab), SA$_6$ (high vaginal swab) and SA$_7$ (endocervical swab) recorded 150.0%, 55.6%, 83.3% and 80.0% loss of resistance markers to erythromycin respectively. Strain SA$_3$ (midstream urine) recorded 50.0% loss (reduction) of resistance markers to gentamycin. Also *Staph.aureus* strains SA$_2$ (ear swab), SA$_5$ (urethral swab) and SA$_7$ (endocervical swab) showed 110.0%, 70.0% and 53.3% reduction in resistance markers to chloramphenicol after treatment with 0.95µg/ml acridine orange. Lastly, strain SA$_4$ (wound swab) recorded 72.7% loss of resistance markers to tetracycline after treatment with 0.95µg/ml acridine orange. Resistance markers present in the seven *Staph. aureus* strains to augmentin, cotrimoxazole and amoxicillin remained unchanged (unaffected) after 0.95µg/ml acridine orange treatment. There was however enhanced improvement in zones of inhibition by strains SA$_1$ (to amoxicillin), SA$_2$ (to cotrimoxazole and amoxicillin), SA$_4$ (to cotrimoxazole and amoxicillin), SA$_5$ and SA$_6$ (to amoxicillin) and SA$_7$ (to cotrimoxazole and amoxicillin).

In Table 7, the summary and distribution of 50.0% and above loss in resistance markers is presented. *Staph.aureus* strains SA$_3$, SA$_7$, SA$_4$, and SA$_1$ recorded 140.0%, 120.0%, 100.0% and 60.0% loss in resistance markers respectively to erythromycin with a mean loss of 60.0%

Table 5. *Staph.aureus* strains that showed ≤50.0% sensitivity enhancement after treatment with 0.75µg/ml dilutions of Acridine orange and incubation at 37^0c for 24hrs.

MDR Strains	Zones of Inhibition (in mm) Around Antibiotic Discs before and After treatment with 0.75ug/ml dilution of Acridine orange							
		AUG	ERY	GEN	CHL	TET	COT	AMX
SA$_1$	Before	0.0	10.0	15.0	12.0	0.0	0.0	0.0
	After	0.0	24.0	19.0	12.0	5.0	0.0	21.0
		(0.0%)	**(140.0%)**	(26.7%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_2$	Before	0.0	12.0	16.0	11.0	9.0	14.0	0.0
	After	0.0	18.0	25.0	11.0	12.0	15.0	10.0
		(0.0%)	**(50.0%)**	**(56.3%)**	(0.0%)	(33.3%)	(7.1%)	(0.0%)
SA$_3$	Before	0.0	5.0	15.0	10.0	0.0	0.0	0.0
	After	0.0	11.0	22.0	20.0	0.0	0.0	0.0
		(0.0%)	**(120.0%)**	(46.7%)	**(100.0%)**	(0.0%)	(0.0%)	(0.0%)
SA$_4$	Before	0.0	9.0	16.0	18.0	11.0	0.0	0.0
	After	0.0	14.0	20.0	21.0	11.0	25.0	23.0
		(0.0%)	**(55.6%)**	(25.0%)	(16.7%)	(0.0%)	(0.0%)	(0.0%)
SA$_5$	Before	0.0	0.0	12.0	10.0	0.0	0.0	0.0
	After	0.0	0.0	21.0	17.0	0.0	0.0	0.0
		(0.0%)	(0.0%)	**(75.0%)**	(70.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_6$	Before	0.0	9.0	13.0	14.0	13.0	0.0	0.0
	After	0.0	11.0	15.0	16.0	17.0	0.0	20.0
		(0.0%)	(22.2%)	**(15.4%)**	(14.3%)	(30.8%)	(0.0%)	(0.0%)
SA$_7$	Before	0.0	5.0	16.0	14.0	12.0	0.0	0.0
	After	0.0	10.0	21.0	23.0	14.0	7.0	9.0
		(0.0%)	**(100.0%)**	(31.3%)	**(64.3%)**	(16.7%)	(0.0%)	(0.0%)

≤50.0% Resistance Marker Losses are highlighted.

Table 6. *Staph.aureus* strains that showed ≤50.0% sensitivity enhancement after treatment with 0.95µg/ml dilutions of Acridine orange and incubation at 37^0c for 24hrs.

MDR Strains	Zones of Inhibition (in mm) Around Antibiotic Discs before and After treatment with 0.95ug/ml dilution of Acridine orange							
		AUG	ERY	GEN	CHL	TET	COT	AMX
SA$_1$	Before	0.0	10.0	15.0	20.0	0.0	0.0	0.0
	After	0.0	25.0	16.0	22.0	7.0	0.0	26.0
		(0.0%)	**(150.0%)**	(6.7%)	(10.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_2$	Before	0.0	12.0	16.0	10.0	9.0	0.0	0.0
	After	0.0	14.0	16.0	21.0	13.0	14.0	18.0
		(0.0%)	**(16.7%)**	(0.0%)	**(110.0%)**	(44.4%)	(0.0%)	(0.0%)
SA$_3$	Before	0.0	0.0	10.0	0.0	0.0	0.0	0.0
	After	0.0	5.0	15.0	20.0	0.0	0.0	0.0
		(0.0%)	(0.0%)	**(50.0%)**	(0.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_4$	Before	0.0	9.0	16.0	20.0	11.0	0.0	0.0
	After	0.0	14.0	20.0	20.0	19.0	25.0	26.0
		(0.0%)	**(55.6%)**	(25.0%)	(0.0%)	**(72.7%)**	(0.0%)	(0.0%)
SA$_5$	Before	0.0	0.0	12.0	10.0	0.0	0.0	0.0
	After	0.0	16.0	13.0	17.0	12.0	0.0	20.0
		(0.0%)	(0.0%)	(8.3%)	(70.0%)	(0.0%)	(0.0%)	(0.0%)
SA$_6$	Before	0.0	6.0	13.0	15.0	12.0	0.0	0.0
	After	0.0	11.0	17.0	16.0	17.0	0.0	21.0
		(0.0%)	**(83.3%)**	(30.8%)	(6.7%)	(14.7%)	(0.0%)	(0.0%)
SA$_7$	Before	0.0	5.0	18.0	15.0	12.0	0.0	0.0
	After	0.0	9.0	21.0	23.0	14.0	7.0	26.0
		(0.0%)	**(80.0%)**	(31.3%)	**(53.3%)**	(16.7%)	(0.0%)	(0.0%)

≤50.0% Resistance Marker Losses are highlighted.

after treatment with 0.35µg/ml acridine orange. This was followed by mean losses of 52.7%, 52.2% and 14.3% to erythromycin after treatment with 0.95, 0.75 and 0.55µg/ml acridine orange respectively. The highest and

Table 7. Distribution of ≤50.0% loss in resistance markers among multidrug resistant *Staph.aureus* strains after treatmentwith 0.35, 0.55, 0.75 and 0.95µg/ml dilutions of acridineorange.

Stap. aureus strain codes	ERY				GEN				CHL				TET			
	0.35	**0.55**	**0.75**	**0.95**	0.35	0.55	0.75	0.95	**0.35**	**0.55**	**0.75**	**0.95**	0.35	0.55	0.75	0.97
SA$_1$	60.0	100.0	140.0	150.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SA$_2$	0.0	0.0	50.0	0.0	0.0	0.0	56.3	0.0	0.0	0.0	0.0	110.0	111.1	66.7	0.0	0.0
SA$_3$	14.0	0.0	120.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
SA$_4$	100.0	0.0	55.6	55.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	72.7	0.0	0.0	72.7
SA$_5$	0.0	0.0	0.0	0.0	116.6	0.0	75.0	0.0	0.0	0.0	70.0	70.0	0.0	0.0	0.0	0.0
SA$_6$	0.0	0.0	0.0	83.3	61.5	0.0	0.0	0.0	0.0	56.3	0.0	0.0	116.7	0.0	0.0	0.0
SA$_7$	120	0.0	0.0	80.0	0.0	0.0	0.0	0.0	0.0	0.0	64.3	53.3	0.0	0.0	0.0	0.0
Mean	**60.0%**	**14.3%**	**52.2%**	**52.7%**	**25.4%**	**0.0%**	**18.8%**	**7.1%**	**0.0%**	**8.0%**	**33.5%**	**33.3%**	**42.9%**	**9.5%**	**0.0%**	**10.4%**

Table 8. Acridine orange dilutions effect on the minimum inhibitory concentration (MIC) of gentamycin usingMDR *Staphylococcus aureus* strain isolated from seminal fluid as inoculums.

Non-Toxic Acridime Orange Dilutions (µg/ml)		New MIC after treatment (µg)	Serial Dilutions of Gentamycin Antibiotic (µg)										Sterile Broth control	Drug control
		80.0	40.0	20.0	10.0	5.0	2.5	1.25	0.64	0.33	Inoculum control		
0.35	2.5	0.0	0.0	0.0	0.0	0.0	0.0	+	+	+	+	0.0	0.0
0.55	No change	0.0	0.0	0.0	0.0	+	+	+	+	+	+	0.0	0.0
0.75	No change	0.0	0.0	0.0	0.0	+	+	+	+	+	+	0.0	0.0
0.95	1.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	+	+	+	0.0	0.0

lowest mean loss of resistance markers therefore, were 60.0% and 14.3% after treatment with 0.35µg/ml and 0.55µg/ml acridine orange respectively. Mean resistance markers reduction of less than 30.0%, 20.0% and 10.0% to gentamycin in were recorded for 0.35, 0.75 and 0.95µg/ml acridine orange treatment. There was no reduction in resistance marker to gentamycin after 0.55µg/ml acridine orange treatment.

Less than 40.0%, 40.0% and 10.0% loss of resistance marker to chloramphenicol was noted after treatment with 0.75, 0.95 and 0.55µg/ml acridine orange respectively with no loss of resistance marker seen with 0.35µg/ml acridine orange treatment. Lastly, less than 50.0%, 15.0% and 10.0% loss of resistance markers to tetracycline was recorded after treatment with 0.35, 0.95 and 0.55µg/ml acridine orange. There was no loss of resistance markers after 0.75µg/ml acridine orange treatment.

In all, mean loss of 50.0% or more of resistance markers was recorded after treatment with 0.35µg/ml (60.0%), 0.95µg/ml (52.7%) and 0.75µg/ml (52.2%) to erythromycin. There was zero loss of resistance markers to gentamycin, chloramphenicol and tetracycline after treatment with 0.55µg/ml, 0.35µg/ml and 0.75µg/ml acridine orange respectively.

Presented in Table 8, is the effect of dilutions of acridine orange on the minimum inhibitory concentration of gentamycin as it inhibited the growth of multidrug resistant *Staph. aureus* (isolated from seminal fluid). Whereas acridine orange dilutions of 0.55µg/ml and 0.75µg/ml yielded no change (i.e. No MIC reduction), 0.35µg/ml reduced the MIC from 10µg to 2.5µg (a four-fold reduction) and 0.95µg/ml reduced gentamycin MIC to 1.25µg (an eight-fold reduction).

DISCUSSION

Byron *et al.* (2003) tested the ability of sesquiterpenoids (i.e. nerolidol, bisabolol and apritone) as well as ethidium bromide to enhance *Staphylococcus aureus* (ATCC 6538) susceptibility to low molecular weight compounds especially antibiotics such as ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and vancomycin. Non-toxic laboratory dilutions (0.35-1.05µg/ml) of homodium bromide have been used to enhance the

sensitivity of a multidrug resistant *Escherichia coli* male urinary tract pure isolate to three commonly used antibiotics which included gentamycin, nitrofurantoin and streptomycin (Otajevwo, 2012).

In this study, dilutions of acridine orange modified according to non-toxic laboratory concentrations of ethidium bromide reported by Wurmb-Schwark *et al.* (2006) were used to treat and reduce resistance markers (≤50.0%) present in multidrug resistant *Staphylococcus aureus* strains isolated from seminal fluid, ear swab, midstream urine, wound swab, urethral swab, high vaginal swab and endocervical 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 chloramphenicol, gentamycin, erythromycin, tetracycline and cotrimoxazole. *Staph.aureus* strains were all completely resistant to augmentin, amoxicillin and cloxacillin (Table 1). The sensitivity of all the *Staph.aureus* strains to the first three drugs is cheering as these drugs are not expensive and also in view of the report that 70-90% of *Staph. aureus* isolates demonstrate wide spread resistance to aminoglycosides, penicillins, macrolides, tetracyclines, lincosamides, cephalosporins, carbapenems, trimethoprim and sulphonamides (Nichols, 1999). Hence, choices can be made between chloramphenicol, gentamycin and erythromycin or a combination of any two in the treatment of infections due to multidrug resistant *Staph. aureus*. The sensitivity profile obtained in this study is subject to verification and confirmation by other researchers. The total resistance recorded against augmentin, amoxicillin and cloxacillin is worrisome because these drugs are used routinely to treat a myriad of human diseases. This same worry with particular reference to augmentin was expressed by Oluremi *et al.* (2011) in their report on antibiograms of uropathogens. 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 (Jan *et al.*, 2004). Based on results obtained, *Staph. aureus* strains isolated from ear swab, wound swab and endocervical swab were resistant to augmentin, amoxicillin and cloxacillin (3 drugs) while *Staph. aureus* strain isolated from high vaginal swab was resistant to four drugs. Strains isolated from seminal fluid and urine were resistant to five drugs each while *Staph. aureus* from urethral swab was resistant to sic drugs (Table 2). 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 *Staph. aureus* in their studies (Gales, 2000; Aiyegoro *et al.*, 2007; Abubakar, 2009). The high

prevalence of multiple antibiotic resistant *Staph. aureus* strains in this study is a possible suggestion that very large population of *Staph. aureus* organisms has been exposed to several antibiotics which is consistent with the report of Oluremi *et al.* (2011).

Acridine orange dilutions of 0.35, 0.55, 0.75 and 0.95µg/ml were used to treat and cure the seven *Staph. aureus* strains with the intent of reducing their resistance markers significantly or eliminating them completely. These dilutions were modified dilutions obtained from the non-toxic laboratory dilutions of Ethidium bromide used and reported by Wurmb-Schwark *et al.*, (2006). Trevors (1986) earlier established non-toxic concentrations of comermycin, rifampicin and novobiocin as curing agents which inhibited DNA gyrase/RNA polymerase thus promoting effective elimination of plasmids.

The loss of 50%-100% of resistance markers after treatment with acridine orange dilutions of 0.35, 0.55, 0.75 and 0.95µg/ml was used as the basis of establishing the curing effects of these dilutions. 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.

Staph.aureus strain SA_1 isolated from seminal fluid recorded 150.0%, 140.0%, 100.0% and 60.0% reduction in resistance markers to erythromycin after treatment with 0.95, 0.75, 0.55 and 0.35µg/ml acridine orange respectively. This somewhat suggests that the highest and lowest loss of resistance markers in *Staph. aureus* strain SA_1 to erythromycin was recorded with 0.95µg/ml and 0.35µg/ml acridine orange treatments. Similarly, loss of resistance markers to erythromycin in *Staph.aureus* strain SA_4 isolated from wound swab after 0.95, 0.75, 0.55 and 0.35µg/ml acridine orange treatments were 55.6%, 55.6%, 0.0% and 100.0% respectively. Resistance marker loss of 50% to erythromycin was recorded for strain SA_2 (ear swab) after 0.75µg/ml acridine orange treatment. At the other concentrations, there was total absence of any loss of resistance marker for *Staph. aureus* strain SA_2. In strain SA_3, over 100% RML was recorded after 0.35 and 0.75µg/ml AO treatment. The pattern was completely different in strains SA_5 and SA_6 as there was absence of RML to erythromycin except for the latter strain which recorded 83.3% RML after 0.95µg/ml AO treatment.

The highest (60.0%) and lowest (14.3%) mean loss of resistance marker were recorded after 0.35µg/ml and 0.55µg/ml acridine orange treatment. The next highest resistance marker reduction was recorded after 0.95µg/ml AO treatment. This finding is agrees with report of Otajevwo (2012) which reported 0.35µg/ml homodium bromide as a significant enhancer of a

multidrug resistant *Escherichia coli* sensitivity to nitrofurantoin, gentamycin and streptomycin. Present study report is also consistent with an earlier report of linkage between acridine orange curing of antibiotic resistance and plasmid borne resistance (Naomi, 1978; Darini, 1996; Adeleke and Odetola, 1997).

Overall mean RML of 60.0%, 52.7%, and 52.2% in all seven *Staph. aureus* strains to erythromycin after AO treatments of 0.35, 0.95 and 0.75µg/ml respectively is consistent with an earlier study which reported significant (P<0.05) sensitivity enhancement (i.e. resistance marker reduction) rates of 171.7%, 138.9%, 57.4% and 50.8% of a multidrug resistant *Escherichia coli* strain after treatment with 0.35, 0.55, 0.75 and 0.95µg/ml homodium bromide respectively following 6, 12, 18 and 24hrs incubation (Otajevwo, 2012).

Findings also suggest 0.35, 0.95 and 0.75µg/ml as potential enhancers of antibiotic sensitivity in multidrug resistant *Staph. aureus* pathogens. Some workers have reported that since many antibiotics are administered together to patient suffering from infections caused by *P. aeruginosa*, simultaneous application of thioridazine (an antipsychotic drug and a non-antibiotic drug) to such patients may be able to eliminate drug resistance plasmids (Mukherjee *et al.*, 2012).

Sensitivity enhancement effect of the subclinical acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin as it affected MDR*Staph. aureus* (isolated from seminal fluid) showed a four-fold (2.5µg) reduction and eight-fold reduction of gentamicin MIC by 0.35 and 0.95µg/ml acridine orange dilutions respectively. The gentamicin MIC remained un-altered after 0.55 and 0.75µg/ml acridine orange dilutions treatment. A similar report had been made by an author which stated that 0.35, 0.45, 0.75, 0.85 and 0.95µg/ml dilutions of homodium bromide reduced the MIC of gentamicin to 2.5µh, 5µg, 5µg, 2.5µg and 2.5µg respectively when tested on an MDR strain of uropathogenic*Escherichia coli* (Otajevwo, 2012). The implication of finding in this study is that when sub-inhibitory doses of either 0.35 or 0.95µg/ml or average of both is/are incorporated into the manufacture of gentamicin (an aminoglycoside) or any other related aminoglycoside and then administered to a patient (diagnosed to be suffering from a disease due to MDR *Staph. aureus* pathogen, a better result in terms of cure of the disease may be achieved as it will require four times its concentration to function invivo. In a related work, Kohler (2010) showed that the resistance of *P. aeruginosa* to tetracycline efflux was reduced from MIC 0.032 to 0.004µg/ml (eight fold reduction) by treatment with phenothiazine. Crowle*et al.* (1992) demonstrated that non-toxic concentrations of phenothiazines in the lung achieved complete elimination of *Mycobacterium tuberculosis.* In another related study, some authors reported the capacity of an aqueous methanolic plant extract -epidiosbulbin E Acetate (EEA) to decrease the

minimum inhibitory concentration of antibiotics against MDR bacteria thus making antibiotic treatment more effective (Shiram *et al.*, 2008).

CONCLUSION

All seven *Staph.aureus* strains used in this study were sensitive to 5(62.5%) of the antibiotics used which included erythromycin, gentamicin, chloramphenicol, tetracycline and cotrimoxazole. The highest and least mean± standard error zones of inhibition which were 17.0±4.9mm and 5.8±4.7mm were recorded by MDR *Staph. aureus* strains SA_4 and SA_5 respectively. *Staph.aureus* strains SA_4 and SA_5 were isolated from wound swab and urethral swab respectively. The highest and lowest mean±S.E zones of inhibition recorded for all the seven strains put together were antibiotic sensitivity reactions to chloramphenicol and cotrimoxazole respectively. Findings therefore suggest the use of erythromycin, gentamicin or chloramphenicol preferably in the treatment of diseases caused by multiple drug resistant *Staph. aureus* pathogens.

Conversely, all seven MDR *Staph. aureus* strains were resistant to augmentin, amoxicillin and cloxacillin as well as cotrimoxazole (septrin). The implication of this resistance profile is that augmentin, amoxicillin and even cloxacillin which are used widely and routinely to treat a gamut of human diseases may not yield good result when used in treatment of diseases due to MDR *Staph. aureus* and therefore alternative drugs of choice should be sought.

Three (37.5%) *Staph.aureus* strains (i.e. SA_2, SA_4 and SA_7) were each resistant to three antibiotics i.e. augmentin, amoxicillin and cloxacillin. Strains SA_6 was resistant to four antibiotics while strains SA_1 and SA_3 were resistant to five antibiotics each. *Staph.aureus* strains SA_5 was resistant to six of the antibiotics used. This particular finding re-establishes the multidrug resistant nature of *Staph. aureus* strains irrespective of source or site they are isolated from. The high prevalence of multiple antibiotic resistant *Staph. aureus* strains in this study therefore is a possible suggestion that very large population of *Staph. aureus* organisms has been exposed to several antibiotics. Symptomatic and asymptomatic patients are advised therefore to desist from self-medication for any justifiable reason. Drugs should be taken based on prescription.

The highest and lowest mean loss of resistance markers (RM) were 60.0% and 14.3% after treatment with 0.35µg/ml and 0.55µg/ml acridine orange (AO) respectively. Mean resistance marker reduction of less than 30.0%, 20.0% and 10.0% to gentamicin were recorded for 0.35, 0.75 and 0.95µg/ml acridine orange treatment respectively for all seven strains. There was no resistance marker loss to gentamycin after 0.55µg/ml

acridine orange treatment. Less than 40.0% and 10.0% loss of resistance marker to chloramphenicol was noted for all strains after treatment with 0.75, 0.95 and 0.55µg/ml acridine orange respectively. With 0.35µg/ml acridine orange treatment, there was no loss of resistance marker to chloramphenicol. Lastly, less than 50.0%, 15.0% and 10.0% loss of resistance marker to tetracycline was recorded after acridine orange dilution treatments of 0.35, 0.95 and 0.55µg/ml respectively. There was no loss of resistance marker to tetracycline after acridine orange dilution treatment of 0.75µg/ml.

In all, mean loss of ≤50.0% resistance marker was recorded for 0.35µg/ml (52.2%) to erythromycin. There was zero loss of resistance markers to gentamicin, chloramphenicol and tetracycline after treatment with 0.55, 0.35 and 0.75µg/ml acridine orange respectively. Findings suggest 0.35 and 0.95µg/ml acridine orange dilutions as potential enhancers of antibiotic sensitivity in multidrug resistant *Staph. aureus* pathogens. That there was zero loss of resistance marker to gentamicin, chloramphenicol and tetracycline even after acridine orange treatment, may be because the genes coding for their resistance markers are chromosome mediated or presence of non-conjugative plasmids.

Sensitivity enhancement effect of the subclinical acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin as it affected MDR *Staph. aureus* strain (isolated from seminal fluid) showed a four-fold and eight-fold reduction in MIC of gentamicin as recorded for 0.35 and 0.95µg/ml acridine orange dilutions respectively. A fast and accurate determination of MIC can ensure optimal effective treatment of patients.

Hence, the application of these findings is that either 0.35 or 0.95µg/mlacridine orange dilution (or their average) when incorporated into the manufacture of gentamicin, the final product could yield an enhanced therapeutic effect in the treatment of diseases caused by MDR *Staph. aureus* pathogens.

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