

Full Length Research Paper

Comparative analysis on the curing effect of acridine orange (mutagen) on beta - lactamase producing *Staphylococcus aureus*, from bovine and human origin

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The effect of the mutagen (acridine orange) on the multi-resistant antibiotics beta lactamase producing *Staphylococcus aureus* strain of bovine and human origin was investigated and tested individually using iodometric (cell supervision) method for the level of beta-lactamase produced by each strain and antibiotic – sensitivity screening using single disc agar diffusion method. Analyses revealed that 80% of the isolates from bovine origin produced beta-lactamase while only 20% showed absence of the enzyme, while 68% of the human isolates produced beta-lactamase while only 32% showed absence of the enzyme sensitivity to cefotaxime, cefuroxime and ceftriaxime were observed in both strains of bovine and human origin. At high concentrations of the mutagen (250 µg/ml), zones of inhibitions were low for penicillin, amoxicillin and augmentine at reference MIC's of 0.25 µg/ml, while the cephalosporins recorded high zones of inhibition at 0.5 and 1.0 µg/ml reference MIC's in all the strains at 250 and 200 µg/ml of the concentration of the mutagen (acridine orange). The resistance bovine strains producing beta-lactamase resisted curing with acridine orange than the human strains of *S. aureus*.

Key words: Acridine orange, *Staphylococcus aureus*, beta-lactamase, iodometric.

INTRODUCTION

Increase tolerance of disinfecting agents can be caused by energy – dependent efflux pumps located in the cell membrane (Surolia and Surolia, 2001). The use of acridine dyes such as acridine orange for curing and recognizing resistant plasmid in resistant *S. aureus* and other bacterial had been reported by Wang and Ripley (1998). Apart from acridine, other agents used for curing are ethidium bromide and mepacrine (Davies and Smith, 1978). Also according to Singh (1972), the genetic effects of acriflavine (an example of acridine dyes) have been studied on two different strains of *Fusarium* (fungi) with regard to photodynamic inactivation and reversion of genetic markers. In the presence of acriflavine (acridine dyes) photodynamic inactivation was observed that it did

not result in a change in the shapes of the survival curves, but induces reversion of the genetic markers in each strain of organisms used. Since the report of elimination of resistant cytoplasmic factors of yeast with acriflavine and acridine orange was made, loss of antibiotic resistance after exposure to a curing agent has been widely accepted as Plasmid mediated resistance (Davis and Smith, 1978). The genes encoding multi drug exported proteins among staphylococci can be divided into two families on the basis of DNA homology and phenotypic properties, (Anthonisen et al., 2002). The *quacA* and *quacB* genes are closely related and differs at the nucleotide level by seven nucleotides. A single amino acid alteration (Ala in *quacB* Asp. In *quacA*, codon 323) is probably responsible for the differences in phenotypic expression, which gives a broader resistance phenotype in isolates harbouring *quacA* genes from *Staphylococcus haemolyticus* (99.9%) identities at the nucleotide level with the same genes from *S. aureus*, demonstrating that

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various Staphylococcal species able to colonize animal and human hosts can exchange the genetic elements involved in resistance to antibiotics and disinfectants (Bjorland et al., 2005). Production of beta-lactamase is one of the mechanisms by which staphylococci become resistance to beta-lactam antibiotics (Anthonisen et al., 2002; Ang et al., 2006). The production of beta-lactamase greatly contributes to the clinical problem of antibiotic resistance (Zhang et al., 2001; Shakibae et al., 2003). Numerous chromosome/and plasmid-mediated type are known. According to Bush et al. (1995), beta-lactamases are enzymes that catalyse the hydrolysis of beta-lactam drugs such as penicillin and cephalosporin. They are up to 190 Beta-lactamase (Bush et al., 1995). According to Bush et al. (1995), beta lactamases are structurally related and probably evolved from enzymes involved in cell wall synthesis, the so called penicillin-binding-proteins (PBP's). The use of antibiotics disinfectants in veterinary practice and animal husbandry may also contribute to the selection and maintenance of resistance factors among *Staphylococcus* spp. (Anthonisen et al., 2002).

The aim of the study is thus, investigating the curing effect of acridine orange (mutagen) on beta-lactamase producing *S. aureus* in bovine and human origin.

MATERIALS AND METHODS

Clinical human isolates were recovered from the routine section of the Medical Microbiology and Parasitology Laboratory, University College Hospital (UCH) Ibadan, Nigeria. While bovine isolates were obtained from the Veterinary Teaching Hospital (VTH), University of Ibadan. All purified isolates (bovine and human origin) were maintained as stock cultures on nutrient agar slants and stored at 4°C. Subculturing was carried out fortnightly to ensure viability of the isolates.

Routine human clinical specimen comprising of skin swabs, wound swabs, (pus/aspirates) and eye swabs were aseptically collected, transported and processed in the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Ibadan. Also specimen from individual cows (bovine) at Veterinary Teaching Hospital (VTH) University of Ibadan, were collected using sterile moistened swab sticks and transferred into tryptone soy broth and subsequently transported to Pharmaceutical Microbiology Laboratory for further culturing, identification and characterization. The clinical sources were nasal exudates (swabs), and breast nipple (swabs).

All suspected colonies of *S. aureus* from the primary culture plates on Blood and MacConkey Agar (Lab. M) were incubated anaerobically for 24 to 48 h at 37°C. After incubation, the plates were examined for appropriate growth characteristics and subsequently numbered with designated code VTH for the bovine and UCH for the human isolates. Yellow mannitol fermenting colonies were confirmed as *S. aureus* by carrying out biochemical reaction test such as positive catalase and tube coagulase test. Grams staining were also carried out according to Ogbulie et al. (1998) and Cheesbrough (2000). A total of 50 isolates were therefore used.

Antimicrobial sensitivity testing

The screening for antimicrobial activity was carried out by the single disc agar diffusion method as described by Singleton (1999). Using

sterile pipette, 0.1 ml of 10^{-2} dilution of an overnight broth culture of each test bacterium was added to 20 ml molten nutrient agar cooked to 45°C, the content was gently swirled to mix before pouring into sterile petri dishes. The seeded culture plates were allowed to set and subsequently dried for 20 min; in the dryer (incubator) at 37°C. After drying, the antibiotic disc were aseptically introduced on the surface of the medium with the aid of the sterile forceps and allowed for 10-15 mins; before incubating at 37°C for 24 h, after which the zones of growth inhibition were determined. Zones less than 14 mm were considered resistant, while those from 14 mm and above were considered susceptible as described by NCCLS (1997) and Taiwo et al. (2005), as standard zones.

Preparation of starch solution and phosphate buffer (0.1M)

The starch solution was prepared fresh as 1% (w/v) aqueous concentration by dissolving 0.25 g of soluble starch in 25 ml of sterile distilled water. The mixture was boiled in an electrothermal water bath (England) with intermittent stirring, to give whitish gelatinous solution, and allow to cool before use.

The preparation of Phosphate Buffer (0.1 m) was carried using potassium dihydrogen phosphate (Analar R-(KH_2PO_4) 1.36 g (Sol. A). Solution B, which is Di-sodium hydrogen phosphate anhydrous (Analar R-(Na_2HPO_4) 1.42 g was dissolved in 100 ml of distilled water, pH 8.8.

The two solutions (A and B) were then mixed using 34 ml of solution A and 66 ml of solution B to give phosphate buffer of pH 7.0-7.3. The buffer was then dispensed in 10 ml, amount into clean universal bottles and sterilized by autoclaving. The solution was used within one week of preparation for the idiometric method of detecting Beta-lactamase production by *S. aureus* strains of both bovine and human origin.

Detection of beta-lactamase production

Overnight nutrient broth culture of each strain was sub cultured by streaking on nutrient agar plate and incubated at 37°C for 18-24 h as described by Sykes (1978), Adeleke and Odelola, (1997). A cell suspension was prepared in triplicates by emulsifying bacterial colonies with a sterile wire-loop in 0.5 ml of freshly prepared phosphate buffered solution containing Penicillin – G (10,000 units). Bacterial cell suspension equivalent to approximately 10^9 cell/ml was prepared for each strain (bovine and human) from an overnight nutrient agar plate culture (Adeleke and Odelola, 1997). The suspension, contained in small sterile test tubes was homogenized on a vortex mixer (Gallen kamp, England) briefly. The standard strains suspension and ordinary penicillin –G phosphate buffered solution serves as controls. The test and control tubes were incubated at room temperature of 26-28°C for a minimum period of 1 h. Thereafter, two drops of freshly prepared 1% aqueous starch solution were added to each suspension. The mixture was shaken gently and briefly, after which one drop of iodine solution was added without shaking the mixture. The mixtures were allowed to stand at room temperature for 10 min. for a colour change from blue or blue to colourless. The result was interpreted as negative, when there was no colour change.

Curing of antibiotic resistance

An overnight culture of each resistant strain of *S. aureus* from bovine and human origin and the control strain was obtained respectively in 10 ml nutrient broth (5 test tubes per strain) containing 250, 200, 150, 100 and 50 $\mu g/ml$ of the mutagen (acridine orange) as described by the modified method of Rotimi

Table 1. Beta-lactamase linked phenotypic resistance pattern of selected antibiotics.

S/N	Resistance type	Resistance phenotypic pattern	Number of resistant strains		Number of strains producing beta-lactamase	
			Bovine (%)	Human (%)	Bovine	Human
1	Single resistance	Pen	25(100)	25(100)	20	17
2	Double resistance	Pen, Amox	22(88)	12(48)	17	9
3	Triple resistance	Pen, Amox, Aug	16(64)	6(24)	11	3
4	Quadruple resistance	Pen, Amox, Aug, CFR	5(20)	3(12)	3	1
5	Pendruple resistance	Pen, Amox, Aug, CFR, CFT	1(4) 0(0)	0	1	0

Pen = Penicillin, Aug = Augmentine, Amox = Amoxicillin, CTF = Cefotaxime, CTR = Cefuroxime.

and Duerden (1981) and Obaseke-Ebor (1988). The mixture was incubated overnight at 37°C for 24 h. After incubation, each mutagen-exposed culture was plated on nutrient agar medium and incubated similarly. Colonies were randomly selected from each of the five plates per strain, for sensitivity testing after growing them overnight in 5 ml nutrient broth (Lab M) and diluted to 10⁻² in sterile distilled water. After shaking, 0.1 ml of the diluted (10⁻²) was seeded into a molten nutrient agar (10 ml), swirled to mix and then poured on a sterile agar plate and allowed to solidify. Wells were made on the set agar medium, 6 mm in diameter, and filled each with reference MIC's of each human *S. aureus*, according to Kucer and Bennet (1979). When necessary, the test concentrations of the antibiotics were increased up to x10 or more. After a pre-incubation diffusion period of 2 h, plates were incubated at 37°C for 24 h and zones examined.

RESULTS

Based on the five selected antibiotics (phenotypic) groups, Analysis revealed that the twenty-five (25) isolates of bovine tested, were all resistant to penicillin-G alone out of which twenty (20) of the strains produced Beta-lactamase enzyme. While 17 strains produced Beta-lactamase out of twenty-two (22) strains resistant to penicillin-G and amoxicillin, only eleven (11) produced Beta-lactamase out of the sixteen strains resistant to penicillin, amoxicillin and augmentine. Five (5) isolates were found to be resistant to penicillin, amoxicillin, augmentine and cefuroxime, but only three (3) produced Beta-lactamas enzyme, while only one (1) bovine isolates found to be resistant to all five (5) selected antibiotics (penicillin, amoxicillin, augmentine, cefuroxime and cefotaxime) produced Beta-lactamase enzyme (Table 1).

Also as stated in Table 1, out of the twenty – five (25) human isolates of *S. aureus* resistant to penicillin alone, seventeen (17) produced Beta-lactamase. While twelve (12) isolates resistant to penicillin and amoxicillin, only nine (9) produced the enzyme. Also six (6) isolates found to be resistant to penicillin, amoxicillin, augmentine, only three (3) produced Beta-lactamase enzyme.

Only one (1) of the isolates produced Beta-lactamase out of the three (3) resistant to penicillin, amoxicillin, augmentine and cefuroxime. None of the human isolates

were found resistant to all the five (5) selected antibiotics tested. All the isolates, including bovine strains were found to be penicillin-resistant strains, but not all the isolates (bovine and human) produced Beta-lactamase enzyme.

Also in the general (multi-drug) phenotypic resistant pattern in relation to Beta- lactamase production in the bovine and human (Table 2) isolates of *S. aureus*. It was shown that, despite the fact that some strains were resistant to more than four antibiotics, they did not produce Beta-lactamase while other strains showed low level of Beta-lactamase production and yet, were resistant to many antibiotics (Beta-lactam) such as the penicillin, amoxicillin, cloxacillin and the augmentine. Also the rate of production was seen to be very rapid when the resistant to antibiotics increased, except in some cases when the resistant to antibiotics were just few. Analysis also revealed that despite resistant to series of antibiotics especially the penicillin, amoxicillin and tetracyclines, no production of Beta-lactamase enzyme was detected in some strains of both bovine and human origin.

Based on the five selected antibiotic the MIC's of the bovine *S. aureus* strains were previously tested, before treating the strains with acridine orange and were observed to be between 2500 to 5000 µg/ml for penicillin, an amoxicillin, augmentine between 156-625 cefuroxime (312-625 µg/ml) an cefotaxime (156-625 µg/ml), which revealed high resistant strains. While the human strains were not as high as the bovine as cefuroxime and cefotaxime recorded between 12.5-50 µg/ml and 12.5-25 µg/ml when the human strains were tested before treatment (Table 3)

Thus, the effect of the mutagen (acridine orange) on the Beta-lactamase producig multi-resistant *S. aureus* strain of bovine and human origin, shows that penicillin could only show a small amount of zone of inhibition on the bovine strains treated even at high concentrations of the acridine orange (250 µmg/ml) at the standard or reference MIC's of 0.25 µg/ml prepared, amoxicillin and augmentine followed the same trend at the same MIC's,

Table 2. Analysis of beta-lactamase production in relation to multi drug resistance strains.

Code of strains		Number of antibiotics type resistant		Rate of beta-lactamase production among strains	
Bovine	Human	Bovine	Human	Bovine	Human
VTH1	UCH210	4	9	P(+)	P(+++)
VTH2	UCH201	6	9	P(++)	Nil
VTH3	UCH212	6	4	Nil	Nil
VTH4	UCH868	9	4	Nil	Nil
VTH5	UCH194	10	1	P(+)	Nil
VTH6	UCH127	8	6	Nil	P (+++)
VTH7	UCH139	9	3	P (+++)	P(+++)
VTH8	UCH142	9	5	P(+++)	P(+)
VTH9	UCH245	8	7	P(+)	P(+++)
VTH10	UCH173	8	7	P(+++)	P(+++)
VTH11	UCH422	6	9	P(+)	P(++)
VTH12	UCH413	6	10	P(+++)	Nil
VTH13	UCH414	5	4	P(++)	Nil
VTH14	UCH612	9	4	P(++)	P(++)
VTH15	UCH313	6	1	P(+++)	P(++)
VTH16	UCH221	6	7	P(+++)	P(++)
VTH17	UCH301	6	3	Nil	P(+)
VTH18	UCH831	4	4	P(+)	P(+)
VTH19	UCH311	5	6	P(+)	Nil
VTH20	UCH331	2	6	P(+++)	P(+)
VTH21	UCH140	7	6	P(+)	P(++)
VTH22	UCH319	10	9	Nil	P(+)
VTH23	UCH340	7	10	P(+)	P(++)
VTH24	UCH700	9	10	P(++)	Nil
VTH25	UCH712	6	7	P(+++)	P(+)

UCH = University College Hospital, VTH = Veterinary Teaching Hospital, P = Positive, Nil = No color change, + = very slow production, ++ = slow production, +++ = rapid production.

Table 3. MIC distribution of bovin/human strains of *S. aureus* before treatment with acridine orange.

Selected drugs	Bovine <i>S. aureus</i>		Human <i>S. aureus</i>	
	MIC µg/ml	No of strains	MIC µg/ml	No of strains
PEN	>5000	25	250	13
			500	12
AMOX	2500	4	250	13
	5000	21	500	12
AUG	156	3	62.5	12
	312	20	125	11
	625	2	250	2
CEFUROXIME	312	14	12.5	5
	625	11	50	20
CEFOTAXIME	156	18	12.5	19
	312	4	50	6
	625	3		

Table 4. Effect of the mutagen (Acridine Orange) on the Multi-Resistant *S. aureus* strains of Bovine Origin 250 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
VTH 1															
S1	10	R	R	10	R	R	8	R	R	18	14	14	18	15	15
S2	10	R	R	10	R	R	8	R	R	18	14	14	18	15	15
S3	9	R	R	10	R	R	8	R	R	18	13	14	19	15	14
S4	10	R	R	10	R	R	9	R	R	17	13	14	18	15	14
Control VTH 1 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
VTH 4															
S1	12	R	R	12	R	R	8	R	R	15	10	10	16	10	10
S2	12	R	R	12	R	R	8	R	R	15	10	10	16	10	10
S3	12	R	R	12	R	R	8	R	R	15	10	10	16	10	10
S4	10	R	R	11	R	R	8	R	R	15	11	10	16	12	10
Control VTH 4 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 5															
S1	7	R	R	10	R	R	8	R	R	18	8	8	24	18	18
S2	7	R	R	10	R	R	8	R	R	18	8	8	24	18	18
S3	7	R	R	11	R	R	8	R	R	19	8	8	24	18	18
S4	8	R	R	10	R	R	8	R	R	18	8	8	24	18	18
Control VTH 5	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 7															
S1	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
S2	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
S3	14	R	R	17	R	R	13	R	R	14	7	8	18	14	14
S4	15	R	R	18	R	R	12	R	R	14	7	7	19	14	14
Control VTH 7	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 10															
S1	15	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S2	15	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S3	14	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S4	14	8	8	15	8	8	12	R	R	25	16	16	25	18	20
Control VTH 10	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, VTH = Veterinary Teaching Hospital, N.T = Not Treated.

except in some cases where zones of inhibition was obtained in strain VTH10 and VTH7 even at 0.03 mg/ml. The case of the cephalosporins were different because, cefotaxime (a third generation Beta-lactamase antibiotics) was observed to record higher zones of inhibition at 0.5 and 1.0 mg/ml standard or reference MIC's prepared in all the selected resistant strains of bovine origin at 250 and 200 µg/ml of the acridine orange used. Cefuroxime

followed the same trend at 250 and 200 mg/ml concentration. Of the mutagen treated strain at reference MIC's of 0.5 and 1.0 µg/ml, except in some case where resistant were observed at reference MIC's of 0.5 at 200 µg/ml of the mutagen concentration on four of the five strains selected for treatment (Tables 4 to 11).

Also the same trend of results on the human strain obtained for penicillin and amoxicillin, except in some

Table 5. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of bovine origin 200 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
VTH 1															
S1	R	R	R	R	R	R	R	R	R	13	R	R	14	7	7
S2	R	R	R	R	R	R	R	R	R	13	R	R	14	7	7
S3	R	R	R	R	R	R	R	R	R	13	R	R	14	7	7
S4	R	R	R	R	R	R	R	R	R	13	R	R	14	7	7
Control VTH 1 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
VTH 4															
S1	8	R	R	R	R	R	R	R	R	R	R	R	12	8	8
S2	8	R	R	R	R	R	R	R	R	R	R	R	12	8	8
S3	8	R	R	R	R	R	R	R	R	R	R	R	12	8	8
S4	8	R	R	R	R	R	R	R	R	R	R	R	12	8	8
Control VTH 4 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 5															
S1	R	R	R	R	R	R	R	R	R	R	R	R	151010		
S2	R	R	R	R	R	R	R	R	R	R	R	R	151010		
S3	R	R	R	R	R	R	R	R	R	R	R	R	151110		
S4	R	R	R	R	R	R	R	R	R	R	R	R	151010		
Control VTH 5	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 7															
S1	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
S2	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
S3	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
S4	15	R	R	18	R	R	12	R	R	14	7	7	18	14	14
Control VTH 7	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 10															
S1	14	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S2	14	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S3	14	8	8	15	8	8	12	R	R	25	16	16	25	18	20
S4	15	8	8	15	8	8	12	R	R	25	16	16	25	18	20
Control VTH 10	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, VTH = Veterinary Teaching Hospital, N.T = Not Treated.

cases on strain UCH173 where zones of inhibitions were recorded at reference MIC's 0.03 and 0.25 µg/ml for penicillin at 250 and 200 mg/ml of the acridine orange concentrations. Augmentine showed a better effect on all the human strains treated with the mutagen at reference Mic's of 0.03 and 0.25 µg/ml at 250 and 200 µg/ml of the mutagen concentrations, but in some cases the effect was only on reference MIC's of 0.25 µg/ml of the augmentine. Almost the same trend of results was

observed for the cephalosporine used, especially the cefotaxime, that had effect on all the treated strains (250 and 200 µg/ml) with the acridine orange at reference MIC's of 0.5 and 1.0 µg/ml. Zone of inhibition were also seen on the human strain treated at 150 µg/ml of the acridine orange, at reference MIC's of 1.0 µg/ml on the treated strains at 250 µg/ml of the curing agent, as in some cases at 200 µg/ml of the mutagen concentration at reference MIC of 1.0 mg/ml.

Table 6. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of bovine origin 150 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
VTH 1															
S1	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
Control VTH 1 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
VTH 4															
S1	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
Control VTH 4 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 5															
S1	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
Control VTH 5	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 7															
S1	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
Control VTH 7	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 10															
S1	R	R	R	R	R	R	R	R	R	15	R	R	14	R	R
S2	R	R	R	R	R	R	R	R	R	15	R	R	14	R	R
S3	R	R	R	R	R	R	R	R	R	15	R	R	14	R	R
S4	R	R	R	R	R	R	R	R	R	15	R	R	14	R	R
Control VTH 10	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, VTH = Veterinary Teaching Hospital, N.T = Not Treated.

The trend of result obtained (zone not up to 14 mm) on some of the Beta-lactam antibiotics in relation to the treated strains with acridine orange (mutagen), shows that the Beta-lactamase enzyme produced may be mediated by plasmids encoded in the *S. aureus* strains.

DISCUSSION

One of the beta-lactam antibiotics, penicillin which has

been reported by several authors and investigators to be the most effective drugs of choice in the Beta-lactam family when it was first introduced (Blondeau and Vaughn, 2000), was found to be highly resisted by the *S. aureus* strains from both the bovine and the human origins in this study. Grouping of five (5) Beta-lactam antibiotics phenotypically base on mode of resistance and Beta-lactamase enzyme production, shows that the bovine isolates produced Beta-lactamase enzyme more than that of the human isolates. Analysis showed that

Table 7. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of bovine origin 100 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
VTH 1															
S1	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
Control VTH 1 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	12	R	R
VTH 4															
S1	R	R	R	R	R	R	R	R	R	R	R	R	11	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
Control VTH 4 (N.T)	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 5															
S1	R	R	R	R	R	R	R	R	R	R	R	R	11	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	11	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	11	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	11	R	R
Control VTH 5	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 7															
S1	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S2	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S3	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
S4	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
Control VTH 7	R	R	R	R	R	R	R	R	R	R	R	R	10	R	R
VTH 10															
S1	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R
S2	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R
S3	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R
S4	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R
Control VTH 10	R	R	R	R	R	R	R	R	R	15	R	R	13	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cef otaxime, VTH = Veterinary Teaching Hospital, N.T = Not Treated.

80% of the bovine isolates produced Beta-lactamase, while 68% produced Beta-lactamase in the human strains. This may be related to overuse of antibiotics in animal feeds and also difference in production system.

The use of certain chemical mutagen such as the acridine dyes sodium dedoecil sulphate and ethidium bromide in effecting curing of antibiotics resistance has been widely accepted as a basis for recognizing R-plasmid in *S. aureus* and other bacteria (Wang and

Ripley, 1998). In this study, acridine orange, a mutagen was used in this respect to verify whether the Beta-lactamase detected were due to mutation, plasmid mediation or chromosomal mediation, as stated by Ambler and Coulson (1991) and Bush et al. (1995), that Beta-lactamase has been found in plasmids and on chromosomes where their expression may be constitutive or inducible. Thus in this study, the inability of augmentine (a Beta-lactamase inhibitor) to inactivate

Table 8. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of human origin 250 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
UCH 210															
S1	8	R	R	18	10	10	8	R	R	15	8	8	18	14	15
S2	8	R	R	18	10	10	8	R	R	15	8	8	18	14	15
S3	8	R	R	18	10	10	8	R	R	15	8	8	18	14	15
S4	9	R	R	18	10	10	8	R	R	16	8	8	18	14	14
Control UCH210(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 868															
S1	10	R	R	12	R	R	8	R	R	14	7	7	18	12	13
S2	10	R	R	12	R	R	8	R	R	14	7	7	18	12	13
S3	10	R	R	12	R	R	8	R	R	14	7	7	18	12	13
S4	10	R	R	12	R	R	8	R	R	14	7	7	18	12	13
Control UCH868(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 139															
S1	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
S2	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
S3	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
S4	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
Control UCH139(N.T)	R	R	R	12	R	R	R	R	R	R	R	R	8	R	R
UCH 142															
S1	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
S2	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
S3	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
S4	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
Control UCH142	R	R	R	8	R	R	R	R	R	R	R	R	R	R	R
UCH 173															
S1	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S2	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S3	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S4	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
Control UCH173	R	R	R	R	R	R	R	R	R	7	R	R	R	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, UCH = University College Hospital, N.T = Not Treated.

the enzyme Beta-lactamase in some case of the bovine strains and few cases of the human strains of *S. aureus*, even after treatment with the mutagen, might be attributed to the Beta-lactamase being chromosomally medicated. Therefore the result of the curing obtained shows that cephalosporins are more effective in the treatment of disease caused by *S. aureus* strains producing Beta-lactamase and generally, for the bovine isolates it may however suggest that chromosomal mediation of the

Beta-lactamase may be playing a role in the bacterial being resistance to the Beta-lactam antibiotics especially penicillin and augmentine (though at higher concentrations of the mutagen). The plasmid-mediated types are usually inactivated by Beta-lactamase inhibitors, such as clavulanic acid unlike the chromosomes types (Richmond and Sykes, 1987). It has also been stated by Taiwo et al. (2003) that 9 (17.7%) of MRSA isolates comprising of 3 (81.3%) of nosocomial isolates and 6(40%)

Table 9. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of human origin 200 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
UCH 210															
S1	8	R	R	14	R	R	R	R	R	12	R	R	18	8	8
S2	8	R	R	14	R	R	R	R	R	12	R	R	18	8	8
S3	8	R	R	14	R	R	R	R	R	12	R	R	18	8	8
S4	8	R	R	14	R	R	R	R	R	12	R	R	18	8	8
Control UCH210(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 868															
S1	8	R	R	10	R	R	7	R	R	12	R	R	18	8	8
S2	8	R	R	10	R	R	7	R	R	12	R	R	18	8	8
S3	8	R	R	10	R	R	7	R	R	12	R	R	18	8	8
S4	8	R	R	10	R	R	7	R	R	12	R	R	18	8	8
Control UCH868(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 139															
S1	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
S2	10	R	R	17	8	8	11	R	R	18	14	14	24	15	15
S3	10	R	R	17	8	8	12	R	R	18	14	14	24	15	15
S4	11	R	R	16	8	8	12	R	R	18	14	14	24	15	15
Control UCH139(N.T)	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
UCH 142															
S1	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
S2	9	R	R	17	12	12	10	R	R	15	R	R	20	14	12
S3	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
S4	8	R	R	18	12	12	10	R	R	15	R	R	20	14	12
Control UCH142	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
UCH 173															
S1	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S2	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S3	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
S4	10	10	10	22	14	14	8	R	R	18	12	12	23	18	17
Control UCH173	R	R	R	R	R	R	R	R	R	7	R	R	R	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, UCH = University College Hospital, N.T = Not Treated.

of community acquired isolates *S. aureus* had no plasmids, but were resistant to some antibiotics, such as penicillin, chloramphenicol and tetracycline. These parameters as well as Beta-lactamase amino acid sequence have been used to classify this enzyme, according to Ambler and Coulson (1990). Plasmid-mediation of Beta-lactamase was also observed to be predominant in the human strains selected, though also noticed at high concentration of the acridine orange (mutagen) used during

the curing process, thus confirming that acridine orange (dye) is a weak curing agent as compared to ethidium bromide (Adeleke et al., 2002) which was not available within the period of this study. To further investigate whether some of the B-lactamase were as a result of plasmid-mediation augmentine which is a Beta-lactamase inhibitor, but could not inhibit most of the Beta-lactamase enzyme produced by the human and bovine strain before being treated with the mutagen, were seen to be effective

Table 10. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of human origin 150 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
UCH 210															
S1	R	R	R	10	R	R	R	R	R	R	R	R14R			R
S2	R	R	R	10	R	R	R	R	R	R	R	R14R			R
S3	R	R	R	10	R	R	R	R	R	R	R	R14R			R
S4	R	R	R	10	R	R	R	R	R	R	R	R15R			R
Control UCH210(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 868															
S1	R	R	R	10	R	R	R	R	R	9	R	R12R			R
S2	R	R	R	10	R	R	R	R	R	9	R	R12R			R
S3	R	R	R	10	R	R	R	R	R	9	R	R12R			R
S4	R	R	R	10	R	R	R	R	R	9	R	R12R			R
Control UCH868(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 139															
S1	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S2	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S3	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S4	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
Control UCH139(N.T)	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
UCH 142															
S1	R	R	R	10	R	R	R	R	R		13R	R10R			R
S2	R	R	R	10	R	R	R	R	R		13R	R10R			R
S3	R	R	R	10	R	R	R	R	R		13R	R10R			R
S4	R	R	R	10	R	R	R	R	R	13	R	R10		R	R
Control UCH142	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
UCH 173															
S1	R	R	R	10	R	R	R	R	R		14R	R	8	R	R
S2	R	R	R	10	R	R	R	R	R		14R	R	8	R	R
S3	R	R	R	10	R	R	R	R	R		14R	R	8	R	R
S4	R	R	R	10	R	R	R	R	R	14	R	R	8	R	R
Control UCH173	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, UCH = University College Hospital, N.T = Not Treated.

on the strains at reference MIC's of 0.25 µg/ml after treating with the mutagen, as against the MIC of 62.5 and 312 µg/ml observed for the human and bovine strains respectively before treatment with acridine orange. According to Anthonisen et al. (2002), the use of antibiotics and disinfectants in veterinary practice and animal husbandry may also contribute to the selection and maintenance of resistance factors among the *Staphylococcus* species. Thus the effect of the acridine

was more on the human strains than the bovine.

Conclusion

This study has confirmed the use of Acridine dye as a mutagen and thus a dye that can be used for detecting the presence of plasmids in highly resistant organisms during analysis. Though acridine orange proved to be a

Table 11. Effect of the mutagen (Acridine Orange) on the multi-resistant *S. aureus* strains of human origin 100 µg/ml of acridine orange concentrations.

Treated strains of <i>S. aureus</i>	PEN reference MIC (µg/ml)			AUG reference MIC(µg/ml)			AMOX reference MIC(µg/ml)			CFR reference MIC(µg/ml)			CFT reference MIC(µg/ml)		
	0.25	0.03	0.03	0.25	0.03	0.03	0.25	0.03	0.03	1.0	0.5	0.5	1.0	0.5	0.5
UCH 210															
S1	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S2	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S3	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S4	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
Control UCH210(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 868															
S1	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S2	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S3	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
S4	R	R	R	10	R	R	R	R	R	8	R	R	10R	R	
Control UCH868(N.T)	R	R	R	10	R	R	R	R	R	8	R	R	10	R	R
UCH 139															
S1	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S2	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S3	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
S4	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
Control UCH139(N.T)	R	R	R	12	R	R	R	R	R	8	R	R	8	R	R
UCH 142															
S1	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
S2	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
S3	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
S4	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
Control UCH142	R	R	R	8	R	R	R	R	R	8	R	R	R	R	R
UCH 173															
S1	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R
S2	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R
S3	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R
S4	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R
Control UCH173	R	R	R	7	R	R	R	R	R	7	R	R	R	R	R

PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFR = Cefuroxime, CFT = Cefotaxime, UCH = University College Hospital, N.T = Not Treated.

weak mutagen, having most of its effects at high concentrations, its usefulness clearly identified the cephalosporines used in this study, such as cefotaxim and cefuroxime to be more potent in treating disease resulting from *S. aureus* infection in veterinary clinics and hospitals. However, the resistances against penicillins were relatively high among *S. aureus* isolates from bovine and human strains at 100%. It is pertinent to mention that the use of antibiotics in bovine feeds should

be curtailed, if not entirely stopped in the dairy industry and farms to prevent unnecessary inactivation of these drugs by the enzymes beta-lactamase.

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