

The level of beta-lactamase linked antibiotic resistance in bovine and human isolates of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus strains from bovine and human clinical sources were investigated. The level of Beta-lactamase mediating antibiotic resistance on them were determine on a comparative basis and the possible drugs of choice for treatment of this disease causing organism, especially those causing mastitis in cow. All the strains were penicillin resistant including the control strains (NCIB8588 and ATCC25923). A total of 20 (80%) of the bovine strains and 17 (68%) of the human were Beta-lactamase producers. The bovine strains recorded high resistance to augmentine 18(72%), erythromycin 22(88%), amoxycillin 22(88%) and cloxacillin 24(96%) when compared to the human strains. All the Beta-lactamase producing strains were sensitive to all cephalosporin class of drugs screened on them. The coefficient of variations from the differences of the means in the individual treatment effect of Beta-lactam drugs used and strain differences at $p > 0.05$ was observed to be 40.79% and 36.89%, with a standard error deviation of $\pm 1.15\text{mm}$ and $\pm 1.29\text{mm}$ for the bovine and human strains respectively. These reveal no relationship between the level or type of Beta-lactamase produced by the bovine and human strains of *S. aureus* with respect to the mode of resistance and rate of the enzyme produced.

Keywords: *Staphylococcus aureus*, Beta-lactamase, iodometric, Beta-lactam, Bovine strain, Human strain, mastitis.

INTRODUCTION

The wide-scale use of antimicrobial drugs has been implicated in microbial drug resistance, which is an adaptive response in which microorganisms become able to tolerate an amount of drug (Bennedsgaard et al., 2006).

S. aureus is a versatile pathogen of man and animals, associated with a wide variety of infections ranging from mild superficial skin infections to life – threatening nosocomial infections, as well as community acquired diseases. *S. aureus* has been isolated from bovine mammary gland suffering from mastitis in a single herd (Bjorland et al., 2005), and is a major pathogen of bovine

mastitis worldwide (Guler et al., 2005). Subtyping bacteria like *S. aureus* is an important epidemiological tool; for example, antimicrobial susceptibility patterns have been used for typing *Staphylococcus* in human medicine (Bjorland et al., 2005; Loir et al., 2003). Mastitis caused by *S. aureus* is the most common types of chronic mastitis and is extremely difficult to control by antibiotics treatment alone. Successful control is gained only through prevention of new infections and cow culling process (Loir et al., 2003). The cure rate after therapy for both clinical and subclinical mastitis has been shown to be lower for β -lactamase-positive *S. aureus* compared to β -lactamase-negative *S. aureus* strains (Bennedsgaard et al., 2006).

Despite implementing intensive control measures, it is difficult to eradicate the intramammary infections caused by this pathogen and it remains a substantial economic problem (Guler et al., 2005). The high rate of resistance

against Beta-lactam antibiotics may have been due to the over use of this drugs in animal feeds and frequent consumption of this animals by man. Previously, several different phenotyping and genotyping techniques have been applied for subtyping of *S.aureus* isolates of bovine and human origin, such as phage typing, plasmid analysis, ribotyping, pulse-field gel electrophoresis, multi locus enzyme electrophoresis and binary typing (Guler et al., 2005). The coagulase protein is an important phenotypic determinant and is accepted as a major virulent factor which has been used to type *S.aureus*, because it encodes a 3'-end variable sequence region (Guler et al., 2005).

According to Ang et al. (2006), antibiotic resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations especially in Nigeria is unknown. The production of Beta-lactamase greatly contributes to the clinical problem of resistance (Zhang et al., 2001; Shakibaie et al., 2003). According to Onyenwe et al. (2011) Beta-lactamases are enzymes that catalyse the hydrolysis of Beta-lactam drugs such as penicillins and cephalosporin. They are up to 190 Beta-lactamases (Onyenwe et al. 2011). Beta-lactamase are structurally related and probably evolved from enzymes involved in their wall synthesis, of the so called Penicillin-Binding-Proteins. Resistant depends on different mechanisms and more than one mechanism may operate for the same antibiotics. Microorganisms resistant to certain antibiotics may also be resistant to other antibiotics that share a mechanism of action or attachment (FVE, 2006).

However, results of susceptibility patterns for commonly used antibiotics indicate that the prevalence of β -lactamase producing *S.aureus* which is resistant to penicillin seems to have remained at a fairly constant level (40–60%) for the last twenty years (Bennedsgaard et al., 2006). Taiwo et al. (2003) stated that *S. aureus* isolated were moderately sensitive to Cefotaxime, Erythromycin and Ciprofloxacin. According to Zhang et al. (2000) and Shakibaie et al. (2003), the production of Beta-lactamase greatly contributes to the clinical problem of antibiotic resistance in plasmid and on chromosomes where their expression may be constitutive or inductive. The organizations for organic agriculture have imposed additional restrictions on the use of antibiotics as an incentive to mitigate the risk of antibiotic resistance and to motivate the farmers to achieve a good herd health without the use of antibiotics. However, it has not been shown whether these initiatives have affected the occurrence of antibiotic resistance in the organic herds (Bennedsgaard et al., 2006).

Thus, the aim of this study is to investigate the type of antibiotics which may be effective and appropriate in the treatment of the diseases caused by this organism, especially to the animals and also to man in a comparable basis, in this part of the world and possibly the level of Beta-lactamase linked or mediating the

antibiotic resistance in both the bovine and human strains of *S.aureus*.

MATERIALS AND METHODS

Clinical human isolates were recovered from the routine section of the Medical Microbiology and Parasitology Laboratory, University College Hospital (UCH) Ibadan. 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 every two weeks 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 samples from individual cows (bovine) at Veterinary Teaching Hospital (VTH) University of Ibadan were collected using sterile moistened swab sticks and transferred into trypton 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). Recommended guidelines were adopted accordingly.

All suspected colonies of *S.aureus* from the primary culture plates on Blood and MacConkey Agar (Lab. M), were incubated anaerobically for 24 to 48hrs 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 as described by Cheesbrough, (2002). 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 in Onyenwe et al. (2011). Using sterile pipette, 0.1ml 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 20mins; 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 10-15mins; before incubating it at 37°C for 24hrs, after which the zones of growth inhibition were determined. Zones less than 14mm were considered resistant, while those from 14mm and above were

considered susceptible as described by Taiwo *et al.* (2003) and CLSI, (2007) as standard zones.

Preparation of starch solution and phosphate buffer (0.1M)

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

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

The two solutions (A and B) were then mixed using 34ml of solution A and 66ml of solution B to give phosphate buffer of pH 7.0-7.3. The buffer was then dispensed in 10ml, 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 subcultured by streaking on nutrient agar plate and incubated at 37°C for 18-24hrs as described by Onyenwe *et al.* (2011). A cell suspension was prepared in triplicates by emulsifying bacterial colonies with a sterile wire-loop in 0.5ml 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 (Onyenwe *et al.*, 2011). 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 1hr. 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 10mins, for a colour change from blue or blue to colourless. The results were interpreted as negative, when there was no colour change.

Statistical analysis

The data from the treatment effect (zone of inhibition) of each of the five (5) selected antibiotics on the twenty-five bovine and twenty-five human isolates of *S.aureus* were compared using the analysis of variance (ANOVA) at $p < 0.05$ levels, in a completely randomized block design with a linear model for the design; $X_{ij} = \mu + t_i + B_j + E_{ij}$ Where B_j = observations made on the j th block(strains), and X_{ij} stands for observation from j th block on the i th treatment using the B-lactam drugs($t=5$, $r=25$), using the method of Martins and Igwemma, (2000).

The null hypothesis, $H_0 = 0$ (no effect), alternative hypothesis, $H_1 \neq 0$ (significant effect)

Coefficient of variation (CV) is given as; $S / X \times 100\%$
Standard Error of the differences between two sample means is given as;

$S_{x_1-x_2} = \sqrt{2S^2 / r}$, S^2 = sample variance which is equivalent to error mean square of the ANOVA.

RESULTS

Among the 50 isolates of *S. aureus* from bovine (25) and human (25) clinical sources, Penicillin-G had the highest number of resistant isolates. Both the bovine and human isolates were sensitive to the Cephalosporine as follows: Cefotaxime (96%), Ceftriaxone (88%) and Cefuroxime 72% for the bovine, while the human had 100% sensitivity for Cefotaxime and Ceftriaxone, thus Cefuroxime had 76%, others were as stated in table1 and 2.

Table 3-6 ,shows that a total of 20 (80%) of the bovine isolates produced Beta-lactamase while only 5 (20%) showed absence of the enzyme out of the twenty bovine isolates that produced the enzyme, 11 (44%) were isolated from the nasal exudates and 9(36%) were from breast swabs (nipple), while only 17 (68%) of the human isolates were found to produce the enzyme Beta-lactamase, while 8(32%) showed absence of the enzyme. Similarly, out of the seventeen isolates of the human that produced the enzyme Beta-lactamase, 5(20%) were isolated from Pyoderma, 11(44%) from wound biopsy and only 1 (4%) from eye swabs (Conjunctivitis). While Table 7 and 8 shows the analysis of variance distribution of the bovine and human strains of *S.aureus*, revealing the source of variation and treatment.

DISCUSSION

Antibiotics resistance is a global public health problem especially those linked to Beta-lactamase production in *S. aureus*. In this study, Beta-lactamase production from the bovine and human origins were observed to be linked

Table 1. Percentage of Antibiotic Susceptibility Pattern of the Bovine *S.aureus* Isolates using Single Disc.

S/N	ANTIBIOTICS	NUMBER OF SENSITIVE ISOLATES (%)	NUMBER OF RESISTANCE ISOLATES (%)	NUMBER OF RESISTANT ISOLATES PRODUCING BETA-LACTAMASE.
1.	PENICILLIN (25 µg)	0 (0%)	25 (100%)	20
2.	CLOXACILLIN (5µg)	1 (4%)	24 (96%)	19
3.	GENTAMICIN (10µg)	20 (80%)	5 (20%)	3
4.	COTRIMOXAZOLE (25µg)	9 (36%)	16 (64%)	12
5.	CHLORAMPHENICOL (30µg)	10 (40%)	15 (60%)	12
6.	AUGMENTINE (30µg)	7 (28%)	18 (72%)	13
7.	AMOXYCILLIN (25µg)	3 (12)	22 (88%)	17
8.	ERYTHROMYCIN (5 µg)	3(12%)	22 (88%)	17
9.	TETRACYCLINE (10 µg)	12 (48%)	13 (52%)	10
10.	CEFOTAXIME (30 µg)	24 (96%)	1 (4%)	1
11.	CEFUROXIME (30 µg)	18 (72%)	7 (28%)	5
12.	CEFTRIAZONE (30 µg)	22 (88%)	3 (12%)	3

Table 2. Percentage of Antibiotic Susceptibility Pattern of the Human *S. aureus* Isolates using Single Disc.

S/N	ANTIBIOTICS	NUMBER OF SENSITIVE ISOLATES (%)	NUMBER OF RESISTANCE ISOLATES (%)	NUMBER OF RESISTANT ISOLATES PRODUCING BETA-LACTAMASE
1.	PENICILLIN (25 µg)	0 (0%)	25 (100%)	17
2.	CLOXACILLIN (5µg)	9 (36%)	16 (64%)	10
3.	GENTAMICIN (10µg)	11 (44%)	14 (56%)	10
4.	COTRIMOXAZOLE (25µg)	3 (12%)	22 (88%)	15
5.	CHLORAMPHENICOL (30µg)	11 (44%)	14 (56%)	10
6.	AUGMENTINE (30µg)	19 (76%)	6 (24%)	3
7.	AMOXYCILLIN (25µg)	13 (52%)	12 (48%)	9
8.	ERYTHROMYCIN (5 µg)	10 (40%)	15 (60%)	11
9.	TETRACYCLINE (10 µg)	4 (16%)	21 (84%)	14
10.	CEFOTAXIME (30 µg)	25 (100%)	0 (0%)	0
11.	CEFUROXIME (30 µg)	19 (76%)	6 (24%)	4
12.	CEFTRIAZONE (30 µg)	25 (100%)	0 (0%)	0

Table 3. Beta-lactamase Linked Phenotypic Resistance Grouping of the Bovine Strains of *S.aureus* on the Selected Antibiotics

RESISTANCE TYPES	RESISTANCE PHYNOTYPIC PATTERN	NUMBER OF RESISTANT STRAINS (%)	NUMBER OF STRAIN OF PRODUCING BETA-LACTAMASE.
SINGLE RESISTANCE	PEN	25 (100)	20
DOUBLE RESITANCE	PEN, AMOX	22 (88)	17
TRIPLE RESISTANCE	PEN, AMOX, AUG	16 (64)	11
QUADRUPLE RESISTANCE	PEN, AMOX, AUG, CRF	5 (20)	3
PENTRUPLE RESISTANCE	PEN, AMOX, AUG, CFR, CFT	1 (4)	1

KEY: PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFT = Cefotaxime, CFR = Cefuroxime.

Table 4. Beta - lactamase Linked Phenotypic Resistance Grouping of the Human Strains of *S.aureus* on the Selected Antibiotics

RESISTANCE TYPES	RESISTANCE PHYNOTYPIC PATTERN	NUMBER OF RESISTANT STRAINS (%)	NUMBER OF STRAIN OF PRODUCING BETA – LACTAMASE.
SINGLE RESISTANCE	PEN	25 (100)	17
DOUBLE RESITANCE	PEN, AMOX	12 (48)	9
TRIPLE RESISTANCE	PEN, AMOX, AUG	6 (24)	3
QUADRUPLE RESISTANCE	PEN, AMOX, AUG, CRF	3 (12)	1
PENTRUPLE RESISTANCE	PEN, AMOX, AUG, CFR, CFT	0 (0)	0

KEY: PEN = Penicillin, AUG = Augmentine, AMOX = Amoxicillin, CFT = Cefotaxime, CFR = Cefuroxime.

Table 5. Frequency of Beta - lactamase Production by the Different Sources of Bovine *S.aureus*

S/NO	SOURCES OF ISOLATES	NUMBER OF ISOLATES (%)	PRESENCE OF BETA – LACTAMASE (%)	ABSENCE OF BETA – LACTAMASE (%)
1.	NASAL EXUDATES	14 (56)	11 (44)	3 (12)
2.	BREAST SWAB (NIPLE)	11 (44)	9 (36)	2 (8)
	TOTAL	25 (100)	20 (80)	5 (20)

Table 6. Frequency of Beta - lactamase Production by the Different Sources of Human *S.aureus*

S/NO	SOURCES OF ISOLATES	NUMBER OF ISOLATES (%)	PRESENCE OF BETA – LACTAMASE (%)	ABSENCE OF BETA – LACTAMASE (%)
1.	PYODERMA	5 (20)	5 (20)	0 (0)
2.	WOUND BIOPSY	18 (72)	11 (44)	7 (28)
3	EYE SWAB (CONJUNCTIVITS)	2 (8)	1(4)	1 (4)
	TOTAL	25 (100)	17 (68)	8 (32)

Table 7. Analysis of Variation (Anova) to Determine the Treatment effect of the B- lactam Drugs on the *S.aureus* Isolates of Bovine

SOURCE OF VARIATION	DEGREE OF FREEDOM(D.F)	SUM OF SQUARE	MEAN SQUARE	F-VALUE
BLOCK	24	989	41.20	2.48••
TREATMENT	4	6790.52	1697.63	102.14••
ERROR	96	1596.12	16.62	
TOTAL	124	9375.64		

KEY: Treatment = B-lactam antibiotics, Block = Replication of zones of inhibition, highly significant = ••

Table 8. Analysis of Variation (Anova) to Determine the Treatment Effect of the B- lactam Drugs on the *S.aureus* Isolates of Human

SOURCE OF VARIATION	DEGREE OF FREEDOM(D.F)	SUM OF SQUARE	MEAN SQUARE	F-VALUE
BLOCK	24	1381.72	57.57	2.72••
TREATMENT	4	5513.72	1378.43	65.29••
ERROR	96	2027.48	21	
TOTAL	124	8922.92		

KEY: Treatment = B-lactam antibiotics, Block = Replication of zones of inhibition, highly significant = ••

to be the major resistant factor for the Beta-lactam antibiotics known as the Beta-lactamase enzymes. Penicillin and amoxicillin used in this study were found to be predominantly less effective on the bovine and human strains of *S. aureus*, followed by augmentin, cefuroxime and cefotaxime in decreasing order. This trend of results supports the work of many authors, for instance, Guler *et al.*, (2005) reported that amongst the *S. aureus* strains isolated from bovine mastitis cases in Turkey, the highest resistance was observed against penicillin, and also that other antimicrobials were usually associated with low resistance, which implies that penicillin cannot be used in the treatment of diseases cause by *S.aureus* in this region. In this study, out of the twenty-five (25) isolates of bovine *S. aureus* screened using single disc diffusion to detect their susceptibility pattern and iodometric suspension method for Beta-lactamase production, all the isolates were resistant to penicillin, recording the highest prevalence ratio of 100%, out of which 80% of the strains produced the enzyme Beta-lactamase. The same trend of result for penicillin was recorded in the human except that 68% of the isolates produced Beta-lactamase enzyme. Cheesbrough, (2002) stated that most strains of *S. aureus* (Particularly hospital strains) are resistant to penicillin due to production of plasmid-coded Beta-lactamase enzyme. Though in this study the Beta-lactamase produced by the organisms were not analyzed whether it was mediated by plasmids. The high rate of Beta-lactamase linked resistance detected in these clinical strains of *S.aureus*, especially in the bovine isolates confirms the relevance of this enzyme in bacterial drug resistance mostly encountered in bovine mastitis. In accordance with the previous study as reported in Taiwo *et al.* (2003) the trend of results in this study reveals that, as far as Beta-lactam antibiotic is concerned, the use of penicillin as the first line of treatment for infection related to *S.aureus* both in man and in animals is a thing of the past, especially in southwest Nigeria. However the frequency of penicillin resistance varies among countries according to Guler *et al.* (2005) who stated that penicillin resistance with an average of 32.4% among *S.aureus* isolates from bovine has been observed in nine European countries and the United States. They stated that penicillin resistance was high among the isolates from Ireland (71.4%), the United

kingdom (67.3%) and the United States (50%) (Guler *et al.*, 2005). Also a report from Denmark, according to Bennedsgaard *et al.* (2006) stated that the proportion of isolates resistant to penicillin was low compared to studies in other countries except Norway and Sweden. Based on the low prevalence of penicillin resistance of *S.aureus* in that region, penicillin should still be the first choice of antimicrobial agent for treatment of bovine intramammary infection in Denmark. Though this view was contrary to what was obtained in this study.

It is also interesting to note, in this study that only one bovine strain found to be resistant to cefotaxime produced Beta-lactamase enzyme. Also the penicillin resistance is higher than the amoxicillin. The high rate of penicillin resistant strain in human isolates in this study also supports the reports in Norway and Sweden, but not Denmark according to Bennedsgaard *et al.* (2006). None of the human strains were resistant to cefotaxime, a third generaion antibiotics, which also agrees with the work of Taiwo *et al.* (2003) that all vancomycon sensitive strains of *S.aureus* were moderately sensitive to cefotaxime (82.4%). Accordingly in this study, the *S.aureus* (human) were highly sensitive to cefotaxime (100%) as compare to bovine strains which gave 96% sensitivity to cefotaxime. However some resistant strains from both sources did not produce Beta-lactamase, which implies that other factors may be responsible for the resistant nature of such organisms, both in bovine and human origins, though the high rate of Beta-lactamase production observe in the bovine penicillin- resistant strains is in line with the reports of Guler *et al.*, (2005).

In this study, only 5(20%) of the bovine strains were found to be negative for the test and 8(32%) for human strains. In this study, patterns of resistance and zones of growth inhibition were tested to verify mode of susceptibility as stated by Loir *et al.* (2003) and Bjorland *et al.* (2005) by measuring the zones of inhibition. These susceptibility pattern support a typing method (Loir *et al.*, 2003; Bjorland *et al.*, 2005) and that not only Beta-lactamase could be linked to bacterial resistance to these drugs but other enzymes too, such as some penicillin-binding proteins (PBP's) or extended spectrum Beta-lactamases.

Zhang *et al.* (2001) reported similar trend of results. In this study the Beta-lactamase may have been induced by

the presence of a given antibiotics. In this study all the cephalosporin screened against *S.aureus* were very active except in some cases for cefuroxime, also 96% of bovine and 100% of human were found to be susceptible to cefotaxime, an extended spectrum Beta-lactam antibiotic (ESBA). Nevertheless some differences were found in erythromycin resistance that it was higher in the bovine (88%), than the human (60%) strain. Thus, these results indicated that Beta-lactamase production may be mediated either by plasmids or chromosomal and that the positive B-lactamase producers were more resistant than the once that are B-lactamase negative.

Based on this observations, analysis of variance was used, The coefficient of variations from the differences of the means in the individual treatment effect of Beta-lactam drugs and the strain differences at $p>0.05$ was observed to be 40.79% and 36.89%, with a standard error deviation of $\pm 1.15\text{mm}$ and $\pm 1.29\text{mm}$ for the bovine and human strains respectively.

CONCLUSION

The high rate of resistance against Beta-lactam antibiotics may be due to the common and uncontrolled use of these groups of antimicrobials, which might be the major selective forces encouraging the development of resistance in bacteria, both in bovine and human origin. Evidently, the drugs used in this study were the most widely used antimicrobial agents for treating mastitis in cows (bovine) or other diseases in man. Thus, this study has clearly shown that these antibiotics could still be used in the treatment of these diseases except penicillin, especially in southwest Nigeria and possibly beyond, because the emergence of antimicrobial resistance globally has not been uniform for all agents, pathogens and within countries of other geographical regions or province. According to World Health Organization (2002), proper routine hand washing after milking in dairy farms, or by hospital personnel, use of safe and aseptic techniques in human, as well as abiding strictly to policy regulating antibiotic prescription and usage, the transmission of these notorious disease causing organisms will be reduced considerably. Nevertheless, the cephalosporin class of drugs is still potent to some varying extent for the treatment of diseases caused by *S.aureus* infection as observed in this study. Absolute care should be taken to avoid increase resistance to these antibiotics as regards to the mode of prescriptions and drug administration.

REFERENCES

- Ang JY, Ezika E, Asmar BI (2006). Antibacterial resistance .Indian J. Paedtr. 71:229-239.
- Bennedsgaard TW, Thamsborg SM, Aarestrup FM, Enevoldsen C, Vaarst M, Christoffersen AB (2006). Resistance to penicillin of *Staphylococcus aureus* isolates from cows with high somatic cell counts in organic and conventional dairy herds in Denmark. Acta Veterinaria Scandinavica. 48: 24.
- Bjorland J, Steinum T, Kvitle B, Waage S, Sunda M (2005). Widespread distribution of Disinfectants resistane genes among *Staphylococci of Bovine* and Caprine origin in Norway. J. Clin. Microbiol. 43(9): 4363-4368.
- Cheesbrough M (2002). Medical Laboratory Manual for Tropical Countries Microbiology. Cambridge University Press Vol. II. pp. 158-159.
- Clinical and Laboratory Standards Institute (2007). Performance Standard for antimicrobial disc susceptibility test. *CLSI document M100-S17, Approved Standard, 7th ed.* 27: 1.
- FVE (2006). Antibiotics resistance and prudent use of antibiotics in Veterinary Medicine. Federation of Veterinary of European. pp. 1-12.
- Guler IOU, Gunduz K, Gular Y, Hadimli HH (2005). Antimicrobial susceptibility and coagulase gene typing of *S.aureus* isolated from Bovine clinical mastitis cases in Turkey. J. Dairy Sc. 88:3149-3154.
- Loir YL, Baron F, Gautier M (2003). *Staphylococcus aureus* and Food poisoning. Genetic Mol. Res. 2(1):63-76.
- Martins OO, Igwemma AA (2000). Applied Statistical Techniques for business and basic sciences. 2nd Ed. Skill mark media Ltd., Owerri, Nigeria. pp. 216-304.
- Onyenwe NE, Mbata, TI, Adeleke, OE (2011). Comparative analysis on the curing effect of acridine orange (mutagen) on beta - lactamase producing *Staphylococcus aureus*, from bovine and human origin. Afr. J. Microbiol. Res. Vol. 5(25): 4278-4290.
- Shakibaie MR, Mansouri S, Hakak S (2003). Research Council of Kerman University of Medical Science, Kerman, Iran J. Med. Sci. 22:1-4
- Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine. 4th ed, JohnWiley and Sons Publisher. pp. 331-337.
- Taiwo SS, Banidele M, Omonigbehin EA, Akinsinde KA (2003). Molecular epidemiology of Methicillin – Resistance *Staphylococcus aureus* in Ilorin-Nigeria, WAJM. 2:100-105.
- World Health Organization (2002). WHO fact sheet, revised Jan. (online). World Health Organization. Geneva Switzerland. No. 194.
- Zhang HZ, Hackbarth CJ, Chansky KM, Chambers HF (2001). A Proteolytic transmembrane siguating pathway and resistance to B-lactam in *Staphylococcus*. National Library of Science. 291 (5510):1962-5.