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Original Article

Prevalence and phenotypic detection of methicillin resistance *Staphylococcus aureus* between ruminants butchered for humanoid intake and animal handlers in Maiduguri, Nigeria

Isa Adamu Gulani, Yaqub Ahmed Geidam, Lawan Adamu, J. R. Lawal and Falmata Ali Abadam

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AFFILIATIONS

- **Isa Adamu Gulani**
- **Yaqub Ahmed Geidam**
- **Lawan Adamu**
- **J.R. Lawal**

Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Borno State, Nigeria.

- **Falmata Ali Abadam**

Department of Food Science and Technology, Ramat Polytechnic Maiduguri, PMB 1070, Borno State, Nigeria.

CORRESPONDENCE

Lawan Adamu
Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Borno State, Nigeria.
E-mail: drlawan3758@unimaid.edu.ng

ABSTRACT

Objective: The objectives of this study was to investigate phenotypically the prevalence of *Staphylococcus aureus* and Methicillin Resistance *S. aureus* (MRSA) in ruminants and animal handlers in Maiduguri, Nigeria.

Materials and methods: A total of 937 samples (900 nasal and skin swabs of cattle and 37 humans samples) were collected in Maiduguri metropolis. The samples were inoculated onto mannitol salt agar (MSA) and blood agar, and the cultrue plates were incubated at 37°C for 24 h. The *S. aureus* colonies showing golden yellow color on MSA were primarily identified as *S. aureus*, which were then subjected for catalase and coagulase tests. All *S. aureus* were finally screened for the presence of MRSA on oxalate resistant screening agar (ORSAB) medium. Antimicrobial susceptibility of the MRSA were measured by disc diffusion method.

Results: The overall prevalence of *S. aureus* was 44.3% (n=414/937). The MRSA could be detected in 12.1% (n=113/937) samples. Considerign different species, *S. aureus* could be isolated from 137 (45.6%), 148 (49.3%) and 119 (39.7%), 11 (29.7%) cattle, sheep, goat and humans, respectively. In ruminants, 27.7% (n-83/300) bulls were positive for *S. aureus*. Similarly, 10.7% (n=32/300) and 22.3% (n=67/300) were positive for *S. aureus* in rams and bucks, respectively. On the other hand, 18% (n=54/300), 38.7% (n=116/300) and 17.3% (n=52/300) samples from cows, ewes and does were positive for *S. aureus*. The highest MRSA could be isolated from ewes (9.3%; n=28/300) follwed by bulls (7%; n=21/300) and bucks (6.7%; n=20/300). In humans, 13.5% (n=5/37) samples were positive for MRSA.

Conclusion: *S. aureus* and MRSA infections in ruminants and animal handlers are documented in this study. The MRSA may exert public health threat to humans.

KEYWORDS

Human, Methicillin resistance, Ruminants, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a microbe of potential public health dread because of its inherent ability to infect and cause life daunting infections and to survive in diverse milieu (Waidvogel, 2000; Pantosti, 2012). The organism is predominantly associated with skin, skin glands and mucous membrane of warm blooded animals as an adaptable microbe and a recurrent invader of animals, as well as humans (Weese et al., 2006a). *S. aureus* is considered nosocomial bacteria incriminated with many infections, including pneumonia, osteomyelitis, endo-carditis, toxic-shock syndrome and food poisoning (Griffeth and Morris, 2008).

It was earlier believed that *S. aureus* from dissimilar reservoirs were host-specific (Sung et al., 2008). However, infections by specific strains of *S. aureus* in domestic and pet animals have been documented to increase in several countries (Francis et al., 2005; Harrison et al., 2014). Hands and surfaces contaminations during scrubbing and extrusion of ruminants are the primary routes of microbes spreading to other food products (Griffeth and Morris, 2008). Ruminants transmit illnesses to man and ruminants are liable to many infections (Rich and Roberts, 2004; Wardyn et al., 2012). The well-being of consuming clean meat from ruminants can be influenced reliably by unsanitary nature of ruminant handlers and contact with unclean surfaces such as worktables and grubby knives (Albuquerque et al., 2007). Enterotoxin secreted by this microbe is accountable for some food poisoning (Griffeth and Morris, 2008) with detrimental public health risk, and causing substantial morbidity and mortality (Waidvogel, 2000; Pantosti, 2012).

S. aureus has the capacity of causing life menacing infections (Waidvogel, 2000; Pantosti, 2012) and the accomplishment resistance to methicillin and to numerous antimicrobial agents. As a result of high mortality caused by the virulent strains of human *S. aureus* penicillin was adapted as a treatment option for skin and soft tissue infections. Identified antimicrobial agents comprises the aminoglycosides, ampicillins, macrolides, tetracyclines fluoroquinolones and chloramphenicol (Lee, 2003; Mamza et al., 2010). Strains of *S. aureus* carrying the *mec*A gene and conspicuously resistant to methicillin, additional β-lactam agents and cefoxitin are mentioned as MRSA (Haran et al., 2012; Harrison et al., 2014).

Epidemiological works on MRSA from human and animal pedigrees divulged that at least some strains have a cross-infection tendencies between animals and humans or the other way round might have transpired (Strommenger et al., 2006; Weese et al., 2006b; Graveland et al., 2011). Lozano et al. (2011) documented that animals could serve as repositories for MRSA infection of humans. The isolation of MRSA from dissimilar species led to the notions that *S. aureus* isolated from dissimilar reservoirs were not specific for host and has been documented in humans by a specific livestock pedigree designated as clonal complex (CC398) or sequence type 398 (ST398) (Huijsdens et al., 2006; Lewis et al., 2008; Smith et al., 2008). MRSA infections in humans have aggravated worldwide public health concern and cognizance of resistant pathogens emanating from veterinary species (Cohn, 2010). Henceforth, rapid isolation in the field and clinical settings is noteworthy in the control of infections emanating from MRSA in animals and humans. It has been accentuated that information on MRSA epidemiology will surely reinforce its effective deterrence and control strategies, including the articulate use of antibiotics. The present study aimed to phenotypically determine the prevalence of MRSA and *S. aureus* in ruminants and animal handlers, in order to evaluate the risk factors as a possible cause of infections and to Screen for antibiotic vulnerability of MRSA isolates and *S. aureus* in cattle, sheep, and goats including in contact animal handlers in Maiduguri, semi-arid zone of Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in Maiduguri metropolis. Maiduguri lies on latitude 11°N and longitude 13.5°E and 35 m above mean sea level, and shares boundaries with Cameroon to the east, Chad republic to the north-east and republic of Niger to the North (**Figure 1**).

Study population: A total of 937 different nasal and skin swabs were randomly collected which comprises of 300 samples each from cattle, sheep and goats and 37 from humans in Maiduguri metropolis abattoir. The swab samples were obtained from Maiduguri Metropolitan abattoir. Permission for the research was granted by University of Maiduguri, Faculty of Veterinary Medicine Animal Utilization Protocol Committee and Borno State Veterinary Department which permitted the research and the sample gathering. Information on the animals was gotten from animal owners who brought these animals for either sale or slaughter at the livestock market and abattoir respectively. The information collected includes the species and breed of the ruminants, antimicrobial agents use and form of grazing.

Bacteriological identification and sampling procedures: Sterilized swabs were used in collecting

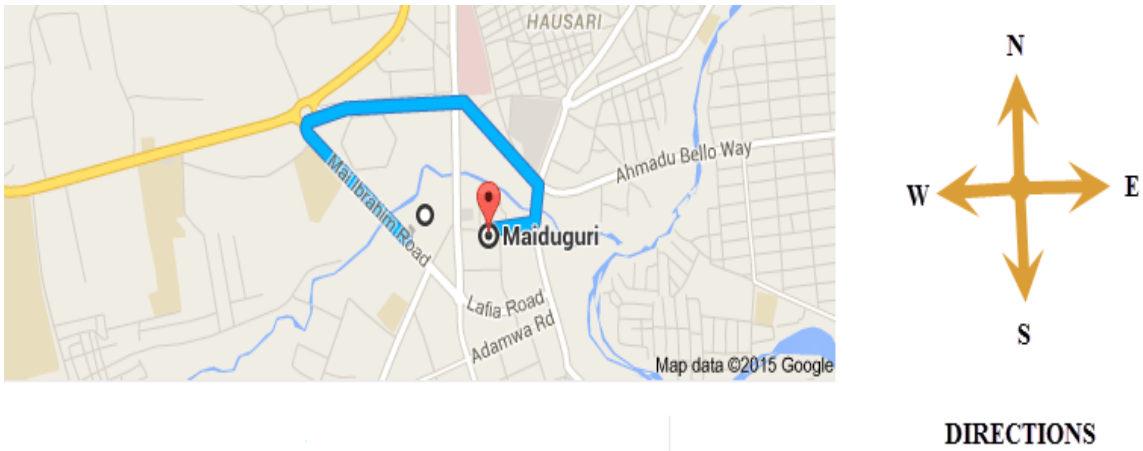

Figure 1. Map of Maiduguri; Source: google maps

samples from the interior of the nasal cavities of ruminants and animal handlers and labeled correctly. The swabs samples were inoculated into mannitol salt agar (MSA) and blood (BA) plates, incubated at 37°C for 24 h. clusters of *S. aureus* with yellowish appearance on mannitol salt agar plates and with haemolysis on blood agar plates were again examined for Gram reaction, tube coagulase, colonial morphology, DNase test and catalase (Cheesbrough, 2006).

Sample collection: The samples were collected by inserting sterile swab into the anterior nares (nostril) and then rotating the swab against the nasal mucosa and also by placing the swab on the skin and then rubbing and rotating it against the skin. The swabs samples were aseptically conveyed to the microbiology laboratory and analysed instantaneously (Cheesbrough, 2006).

Identification and isolation of bacteria: Swabs samples collected was cultured on mannitol salt agar (selective media for *S. aureus*) according to the conventional technique. The culture plates were incubated at 37°C for 24 h. The suspected *S. aureus* colonies (golden yellow colonies) showing mannitol fermentation were recognized by means of conventional microbiological techniques including catalase, colonial morphology and coagulase test which were carried out as previously described by Cheesbrough (2006).

Preparation of Oxacillin resistant screening agar base (ORSAB) medium: Suspend 51.75 gm in 500 mL of distilled water and bring gently to boil to dissolve. Sterilize by autoclaving at 121°C for 15 min. Cool to 50°C and aseptically add the content of one vial of ORSAB selective supplement (SR195E) reconstituted as directed, mix well allow to solidify (Oxoid, UK).

Antibiotic sensitivity tests: *S. aureus* species examined for antibiotics susceptibility to determine methicillin resistance by disc diffusion technique by means of Muella-Hinton agar (**Figure 3**) in accordance to the Clinical and Laboratory Standard Institute guidelines (CLSI, 2007). The following antimicrobials impregnated discs were used: Erythromycin (15 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Oxacillin (1 µg), Clindamycin (2 µg), Cephazolin (30 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Tetracycline (30 µg), Sulfamethoxazol/trimetho-prim (25 µg). The results were interpreted according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2007).

Data analysis: The obtained data from the study were analyzed using prevalence (%) and 95% Confidence interval to determine the differences in proportion and statistical significance between variables at $P<0.05$ with JMP version 11 Statistical Software (SAS Institute Inc., Cary, NC).

RESULTS

A total of 937 samples were analyzed which comprises of 300 samples each from cattle, sheep and goats, and 37 were human. Among them, 137 (45.7%) cattle, 148 (49.3%) sheep, 119 (39.7%) goats, and 11 (29.7%) humans were positive for *S. aureus*, respectively. The overall prevalence was 44.3% (**Table 1**).

Table 2 shows the result of morphological and biochemical characteristics of *S. aureus* in the study. Overall, 45.4% isolates were positive for catalase, 44.3% samples were positive for coagulase, and 12.1% showed intense blue coloration on ORSAB media growth.

Table 1: Prevalence of *S. aureus* based on species of animals

Animals	Samples (n)	Positive (n)	Prevalence (%)	95% confidence interval	
				Lower	Upper
Cattle	300	137	45.7	0.4012	0.5133
Sheep	300	148	49.3	0.4372	0.5496
Goat	300	119	39.7	0.3430	0.4530
Human	37	11	29.7	0.1749	0.4578
Total	**937**	**415**	**44.3**		

Table 2: Result of Biochemical test screening for *S. aureus*

Biochemical Test	Positive Test
Catalase	425 (45.4%)
Coagulase	415 (44.3%)
ORSAB	113 (12.1%)

Table 3: Prevalence of MRSA and *S. aureus* isolated from the skin and nasal of ruminants

Animals	*S. aureus*		MRSA	
	Nasal prevalence (%)	Skin prevalence (%)	Nasal prevalence (%)	Skin prevalence (%)
Ruminants	70 (72.16)	27 (27.84)	58 (71.6)	23 (28.4)
95% C.I	0.6253, 0.8010	0.1990,0.3447	0.6098, 0.8027	0.1973, 0.3902

C.I = Confidence interval; MRSA = Methicillin-resistant S. aureus (MRSA)

Table 4: Prevalence of MRSA in ruminants and humans

Animal species	MRSA	%	95% confidence interval	
			Lower	Upper
Cattle (n=300)	37	12.3	0.0908	0.1653
Sheep (n=300)	32	10.7	0.0766	0.1467
Goat (n=300)	39	13.0	0.0966	0.1728
Humans (n=37)	5	13.5	0.0591	0.2797
Total (n=937)	**113**	**12.1**		

Table 5: Gender prevalence of *S. aureus* and MRSA isolate from cattle, sheep, goats and humans

Animals	Sex	Prevalence (%) of *S. aureus*	95% C.I of *S. aureus*		Prevalence (%) of MRSA	95% C.I of MRSA	
			Lower	Upper		Lower	Upper
Cattle	Bulls	83 (27.7)	0.2291	0.3299	21 (7.0)	0.0462	0.1046
	Cows	54 (18.0)	0.1407	0.2274	16 (5.3)	0.0331	0.0848
Sheep	Rams	32 (10.7)	0.0766	0.1467	4 (1.3)	0.0052	0.0337
	Ewes	116 (38.7)	0.3334	0.4429	28 (9.3)	0.0653	0.1315
Goat	Bucks	67 (22.3)	0.1798	0.2738	20 (6.7)	0.0436	0.1008
	Does	52 (17.3)	0.1347	0.2202	19 (6.3)	0.0409	0.0968
Human	Men	11 (29.7)	0.1749	0.4578	5 (13.5)	0.0591	0.2797
Total		**415 (44.3)**			**113 (12.1)**		

Table 6: Antibiotic susceptibility of MRSA isolate from cattle, sheep, goats and humans

Antibiotics	Susceptible animals	Moderately susceptible animals	Resistant Animals
Cephazatin	81 (71.7%)	0 (0%)	31 (27.0%)
Sulphamethoxazol	73 (64.6%)	22 (19.5%)	18 (15.9%)
Oxacillin	0 (0%)	23 (20.6%)	0 (79.7%)
Ciprofloxacin	113 (100%)	0 (0%)	0 (0%)
Chloramphenicol	92 (81.4%)	11 (9.7%)	10 (8.9%)
Erythromycin	27 (23.9%)	20 (17.7%)	66 (58.4%)
Tetracycline	23 (20.6%)	34 (30.0%)	56 (49.6%)
Clindamycin	26 (23.0%)	39 (34.5%)	48 (22.5%)
Gentamycin	113 (100%)	0 (0%)	0 (0%)
Cefoxitin	0 (0%)	3 (2.7%)	110 (97.4%)

Table 3 shows the result of *S. aureus* and MRSA isolated from ruminant which were characterized phenotypically in the study. Overall, 415 (44.3%) *S. aureus* were detected (**Table 1**). Upon inoculation of these 415 *S. aureus* isolates, on ORSAB media, 113 (27.3%) isolates showed intense blue coloration and were considered as MRSA (**Figure 2**). The result showed that from the 97 *S. aureus* detected, 70 (72.16%) were detected from nasal region

while 27 (27.84%) were from the skin. On the other hand, 58 (71.6%) MRSA could be detected from nasal region while 23 (28.4%) were from the skin.

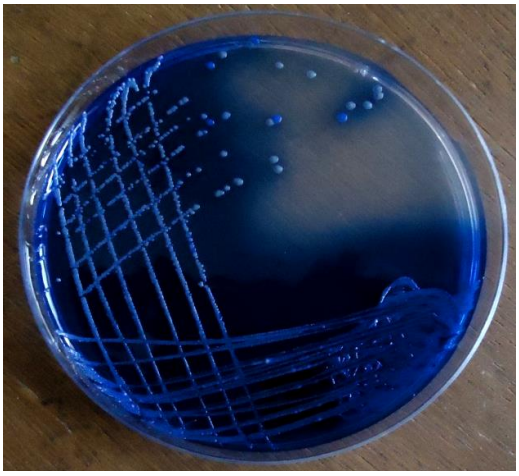

Figure 2. Intense blue coloration considered MRSA

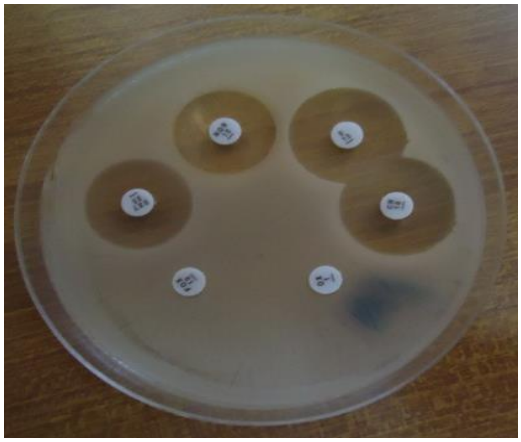

Figure 3**:** Antibiotic sensitivity tests

Table 4 shows the result of the prevalence of MRSA in ruminants and humans in the study area. Out of the total sample tested, 113 (12.1%) samples that were ORSAB positive, 37 (12.3%) tested positive for MRSA in cattle, 32 (10.7%) tested positive for MRSA in sheep and 39 (13.0%) tested positive for MRSA in goats while 5 (13.5%) tested positive for MRSA in animal handlers.

Table 5 shows the result of prevalence of *S. aureus* and MRSA isolates from cattle, sheep, goats and humans according to sex. Of the 528 samples, 415 (78.6%) of the samples were positive for *S. aureus* and 113 (21.4%) were positive for MRSA. In the population of cattle, the male and female with 83 (27.7%) and 54 (18.0%) respectively tested positive for *S. aureus* while those that tested positive for MRSA in the male and female cattle were 21 (7.0%) and 16 (5.3%) respectively. In the population of sheep, the male and female with 32 (10.7%) and 116 (38.7%) respectively tested positive for *S. aureus* while those that tested positive for MRSA in the male and female sheep were 4 (1.3%) and 28 (9.3%) respectively. In the population of goats, the male and female with 67 (22.3%) and 52 (17.3%) respectively tested positive for *S. aureus* while those that tested positive for MRSA in the male and female goats were 20 (6.7%) and 19 (6.3%) respectively. In humans, the male had 11 (29.7%) that were positive for *S. aureus* while those positive for MRSA in the male were 5 (13.5%).

Table 6 shows the results of antibiotic susceptibility of MRSA isolate from cattle, sheep, goats and handlers. The result of antibiotic susceptible testing by NCCL method indicated that the isolates were highly susceptible to chloramphenicol, Cephazatin, ciprofloxacin, sulphamethoxazol erythromycin, Tetracycline, clindamycin and gentamycin. Are moderately susceptible to sulphamethoxazol, Oxacillin and clindamycin. There was resistance against cefoxilin, tetracycline, erythromycin and oxacillin.

DISCUSSION

The study provides an evidence of 44.3% overall prevalence of MRSA in ruminants and humans (animal handlers) in Maiduguri. From the samples examined, prevalence of 45.7% was found in cattle; 49.3% in sheep; 39.7% in goats, and 29.7% in humans. These results indicated a considerable existence of *S. aureus* and MRSA among livestock and livestock handlers. *S. aureus* and MRSA showed significant differences between both sexes of ruminants and human. The findings of this study was in consistent with the report of Jones et al. (2002).

This present study showed that the samples that were positive for *S. aureus* and MRSA were mostly orginated from nasal and skin sources. The distribution of MRSA and *S. aureus* were higher in the nasal samples as compared to those from the skin. The fact that *S. aureus* is ubiquitous, air borne and can be transmitted through aerosol makes this nose and skin the primary invaded sites of the organism in most instances. This finding was in agreement with the report of Mai-siyama et al. (2014) and also quite analogous to the conclusions arrived by Loeffler et al. (2010) who documented on cases of MRSA in different species of animal at a veterinary clinic. The disparity may be as a result of the differences in the design of study, study environment, sample size, type of sample collected, and method of *S. aureus* isolation and use of antibiotics.

Nevertheless, the present study is in accord with consistent surveillance findings in small animals of MRSA in the US showing that MRSA has been investigated to

increase exponentially (Jones et al., 2002). Infections of MRSA progresses, with diverse forms and associated clinical problems were documented. Sufficient information on risk factors and control of infection method within hospital and public setting is of supreme prominence (Mai-siyama et al., 2014). The development of LAMRSA has sufficiently added extra dimension to the comprehension of MRSA infections. In the third worlds, predominantly the sub-Saharan Africa with scarcity of information on MRSA infections, imminent data are required from animal and human population. To our understanding, this is the first documentation of MRSA infection among ruminant butchered for humanoid intake and animal handlers in Maiduguri, Nigeria. The principal findings of our research are, (i) MRSA infection among the ruminant and animal handlers, (ii) antibiotic susceptibility form of *S. aureus* isolates and (iii) the demographic variates related with MRSA infection. Therefore, the findings has created awareness on LA-MRSA infection in the study area including food safety and public health threats, which is in consonants with the findings of Lee (2003).

In the present study, infections of MRSA and MSSA isolates were lower as compared to *S. aureus*. The low MRSA and MSSA colonization rate is similar to the reports of Alzohairy (2011) and Gharsa et al. (2012). MRSA infections fluctuate in animals depending on ecological locations; in a research carried out in Saudi Arabia, high MRSA infections was documented amongst camels and cattle as compared to our findings on cattle and sheep (Alzohairy, 2011, 2012). Variable rate had been stated in France and Tunisia (Vautor et al., 2005; Gharsa et al., 2012), nevertheless, MRSA infections in this research is similar to the level documented by Stastkova et al. (2009) in Poland.

The high MRSA and MSSA infections recorded amongst the cattle in the present research, is of public health threat, because cattle has the highest number of ruminant raised within the public in the study area and they are the main source of proteins. The motives considered as public health threats are- (i) possibility of dissemination and transmission of MRSA isolates occurring through contact including propinquity through domestication and rearing, (ii) nasal discharge on transit and (iii) pollution of meat and milk products by infected handlers. This observation is in line with similar report of public health hazards attributed to handling of animals with MSRA (Lee, 2003; Mai-siyama et al., 2014). Studies have documented that MRSA infections in cattle modeled an impending risk of 60% transmission to animal handlers (Lee, 2003; Graveland et al., 2011).

In Nigeria, the forefront antibiotics regularly used in human and veterinary medicine are erythromycin, tetracycline, penicillin and some quinolones. In the present study, amongst the antimicrobial agents verified, MRSA and MSSA isolates established high degree of sensitivity on some aminoglycosides like streptomycin, amikacin and tobramycin. This form divulged that these agents are still effective for staphylococcal infections therapy and management, but also as ancillary preference for the therapy of livestock infection due to multidrug resistant of *S. aureus* strains in veterinary settings (Mai-siyama et al., 2014). In addition, *S. aureus* strains (2 MRSA from cattle and 4 MSSA from sheep) in the present study established inducible phenotype, the form is in consonant with other studies predominating in MSSA as compared to MRSA (Levin et al., 2005; Alzohairy, 2012; Chandrasekaran et al., 2014). As accessible internationally, macrolides are forefront antibiotics extensively used for the therapy of human and animal infections. Widespread usage of these antibiotics results in the assortment of resistant bacteria and genetic determinants of resistance can be conveyed from animals to humans via foodstuffs (Schlegelová et al., 2004). D-test is used to validate the inducible and constitutive phenotype, and thus, determine the possible chemotherapeutic failure (Levin et al., 2005).

Though, surveillance research are encouraged universally to provide information on LA-MRSA, but there are constraint, predominantly in comparison with epidemiological information. These constraints comprises of low class standardization in the procedure employed, in addition, *Staphylococcus* spp., like *S. schlieferi, S. intermedius*, *S. hyicus*, *S. pseudointermedius and delphini* released positive tube coagulase result that may be identified as MRSA, chiefly in low-class laboratory in which examinations are based on phenotypic classification (Morgan, 2008).

CONCLUSION

The MRSA infections in ruminants and animal handlers found in this study may escalate the resistance which is of great public health concern. The motive could be of transmission and dissemination of these resistant strains and genes between animals and human, and successively passing it to the food chain. The situation could deteriorate if apposite measures are not put in place. *S. aureus* and MRSA were detected in Maiduguri, Borno State. Furthermore, there is significant changes in the dissemination of *S. aureus* and MRSA in the nasal and skin regions in addition to the sexes of the ruminants and male animal handlers. Nevertheless, the present study indicated MRSA an evolving pathogen in ruminants and

human in Maiduguri, Borno State. Even though the size of the sample was small, it might perchance offer the prevalence of MRSA and *S. aureus* in ruminants and animal handlers in Maiduguri.

CONFLICT OF INTEREST

Nothing to declare.

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