Nasal Carriage of Methicillin Resistant Staphylococcus Aureus in Livestock and Farm Workers in Two Communities in Lagos, Nigeria

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ABSTRACT

The epidemiology of methicillin resistant *Staphylococcus aureus* (MRSA) is dynamic and the associated public health risk is likely to increase in settings where there is close interaction between humans and animals. This study assessed the occurrence of antimicrobial resistance among *S. aureus* from livestock and farm personnel, and estimated methicillin-resistance among the isolates. Two hundred and fifty (250) nasal specimens were collected from sheep, goats, cows and farm personnel who had contact with the animals in two farms in a sub-urban region within Lagos State. Antibiotic susceptibility test was performed using disk diffusion method. The presence of *mec*A and *mec*C gene was determined by PCR. *S. aureus* was recovered from 141 (56.4%) of the 250 nasal samples analyzed: 32 (22.7%) from cows, 25 (17.7%) from sheep, 32 (22.7%) from pigs, 24 (17.0%) from goats and 28 (19.9 %) from farm workers. Ten isolates, consisting of 4 from cows (8%), 3 from pigs (6%), and 3 from farm personnel (6%), were positive for MRSA. The human-MRSA were recovered from pig workers (2) and a cow farm worker (1). All MRSA strains were resistant to at least three different antimicrobial categories. The MSSA strains were classified into thirteen antibiotypes of various subtypes. *mec*C – MRSA was not detected. The high frequency of *S. aureus* with phenotypic multidrug resistance traits encountered in this study presents a major public health issue. Thus, practices directed at minimizing the burden of antimicrobial resistance in farm animals should be initiated.

Keywords: Colonization; Farm Personnel; Livestock; *Staphylococcus aureu*s

INTRODUCTION

Staphylococcus aureus has been implicated in a variety of infections in humans and animals. The organism represents a serious public health burden in hospital and community settings, as well as an economic and animal welfare problems in farm animals. It is a major cause of *S. aureus* mastitis in cows (1, 2) and may lead to great economic losses in the dairy industry. For chickens, *S. aureus* is associated with severe infections including comb necrosis, bacterial chondronecrosis with osteomyelitis, septicaemia and "bumble foot" (3, 4). Pigs are often carriers and rarely infected (5), but have the capability for disseminating the pathogen in the general population. In human, the bacterium is often carried asymptomatically at various body sites and such carriage could increase the risk of developing infections as well as assist in spreading the pathogens to other humans. It is a notable cause of skin and soft tissue infection, bacteraemia and food poisoning in humans (6).

The persistence and virulence of *S. aureus* are consequences of its increased antibiotic selective pressure. Methicillin-resistant *S. aureus* (MRSA) is the most frequently

described antibiotic resistant strain with ability to acquire resistance to various classes of antibiotics and virtually to all β-lactam antibiotics (7). For the past 25 years, high dominance of MRSA has been consistent in hospitals globally (8). Furthermore, large reservoirs of Community-associated (CA) MRSA strains existing outside health care facilities are increasingly reported as significant pathogens in many countries (9). However, a recent shift in MRSA epidemiology involves its emergence in animals including pets and livestock (10). This variant designated as Livestock-associated MRSA (LA-MRSA) was discovered in early 2000 among pigs and pig farmers in the Netherlands (11). Ever since, a considerable number of studies in several European countries, USA and Asia have focussed on LA-MRSA (11, 12). Other reservoirs of LA-MRSA include cattle, poultry and wild animals (10, 13).

Like other MRSA variants, LA-MRSA is assumed to be multidrug resistant in nature, displaying resistance to ≥3 antimicrobial classes in addition to β-lactams (14, 15). It differs from HA-MRSA and CA-MRSA in its genomic composition (16) and could harbour the *mec*A homologue or the *mec*C (originally called *mec*A$_{LGA251}$), which has 70% similarity to *mec*A at the DNA level (17). Generally, the *mec* gene encodes for an altered form of penicillin-binding protein (PBP), which has a reduced affinity for beta-lactam antibiotics. At amino acid level, *mec*C encodes PBP2c and has about 63% amino acid homology with PBP2a (17). Basically, *mec*A – positive MRSA strains have been detected in animals, humans and the environment and they may harbor virulence genes which can complicate the development and management of infections (6). Similarly, human acquisition of MRSA carrying *mec*C could occur as a result of direct contact with livestock, contaminated environment or food products (18). Its zoonotic transmission potential was reported in Spain where a patient with *mec*C-positive MRSA sepsis died in the emergency department (19). In another study, Petersen *et al*. (20) provided evidence of transmission and development of infections in two persons who had contact with cows and sheep carrying *mecC* MRSA strains. The authors inferred that *mec*C-carrying MRSA can be exchanged between humans and ruminants.

In Nigeria, *mec*A-MRSA colonisation and infection is a widespread problem and a large number of studies have focused on the phenotypic as well as genotypic analyses of human *S. aureus* isolates (21-22). In addition, the use of antimicrobial agents in animal production is a common phenomenon (23). In the South-East region of the country, the antibiotic susceptibility test of *S. aureus* from goats, sheep, cow, pigs and rabbits revealed that 79% of the *S. aureus* strains were resistant to one or more of the antimicrobial agents tested (24). Also, examination of *S. aureus* from bovine mastitic milk in the North-Central region of the country revealed that only two out of the twenty six (7.6%) isolates examined were positive for *mec*A-MRSA (25). However, much less is known about the animal reservoirs of MRSA in the South-western region.

Lagos, a metropolitan city, is characterised by intensive livestock (especially poultry and pigs) farming practices in the sub-urban region. This could be a huge problem considering recognition and spread of the new MRSA strains among food animals and their human contacts worldwide (26). Therefore, we were prompted to evaluate the presence of MRSA in cattle (sheep, goats, and cows), pigs and their human contacts in two farms located in the sub-urban region of Lagos State. We aimed to determine the antibiotic susceptibility patterns of the isolates and the occurrence of *mec*A- and *mec*C-MRSA strains.

MATERIALS AND METHODS

Study Area and Sampling

We studied two livestock hubs in two communities in Lagos, Nigeria. Both sites are approximately 23.8 km apart. The centers were selected due to cattle and pigs rearing activities. Although, information on the total population of the livestock on the farms was not ascertained, management operations are based on private-public partnership between the State and individuals. Samples were collected based on consultation and consents of the personnel who had direct contacts with the animals. Furthermore, permission was obtained from the Lagos State Ministry of Agriculture prior to sampling. Nasal samples were randomly collected from two hundred (200) animals and fifty (50) farm workers. The farm workers assisted with the collection of samples from the animals. The procedure for specimen collection was non-invasive and there was no observable scar inflicted on the subjects during the process. All human participants signed informed consent forms. All processes were closely supervised by one of the investigators.

Bacterial Isolation and Identification

The nasal swab was collected from both nostrils of each animal and participating farm personnel using sterile cotton-tipped swabs. Specimens were transported immediately to the Microbiology laboratory. The swabs were incubated for 24h at 37°C in brain heart infusion broth (Oxoid, UK) and then streaked onto mannitol salt agar and incubated at the same conditions. Colonies were purified and stored at -20°C in 10% glycerol-brain heart infusion broth for subsequent analysis. Presumed colonies of *S. aureus* were identified by morphological characteristics, Gram staining reaction, catalase, coagulase (slide and tube), polymixin B susceptibility, DNase production, test with novobiocin disk, oxidase, maltose and trehalose fermentation tests (27). A control strain was included in all the biochemical tests.

Antibiotic Susceptibility Testing

The disk diffusion method using Muller-Hinton agar was used to determine the susceptibility patterns of the isolates as recommended by The European Committee on Antimicrobial Susceptibility Testing (28). The antibiotic disks used were: tetracycline (TET) 30µg, gentamycin (GEN) 10µg, erythromycin (ERY) 5µg, fusidic acid (FUS) 10µg, ciprofloxacin (CIP) 5µg, trimethoprim-sulphamethoxazole (SXT) 25µg, amoxicillin/clavulanate (AMC) 30µg, cefuroxime (CRX) 30µg, ceftriaxone (CTR) 30µg, and ceftazidime (CAZ) 30µg. The antibiotics tested included a variety of antibiotic families and the selection was based on common use without veterinary consultation by livestock farmers as well as regional use in human medicine. Methicillin resistance was phenotypically assessed using 1*µg* oxacillin (OXA) and 30µg cefoxitin (FOX) disk diffusion method (29). *S. aureus* isolates were classified as MRSA if the inhibition zone was less than or equal to 21 mm for cefoxitin or less than or equal to 10 mm for oxacillin. All antimicrobial agents were obtained from Oxoid, UK. A methicillin susceptible *S. aureus* strain (ATCC 29213) and methicillin resistant *S. aureus* strain (ATCC 33591) were used as negative and positive control respectively for the susceptibility tests. Multidrug resistance was determined according to the definition of Magiorakos *et al.* (30).

Amplification of mecA and mecC genes

Due to high cost and the logistical difficulties, 50 isolates with resistance to all the beta-lactams and cephalosporins were subjected to uniplex PCR for the presence of *mec*A *and mec*C genes. Chromosomal DNA was extracted by boiling (31) and amplification was carried out using previously described DNA sequences. For *mec*A, the following primer sets were used: *mec*AP4F (5'-TCCAGATTACAACTTCACCAGG-3') and *mec*AP7R (5'-CCACTTCATATCTTGTAACG-3') (32). The primers, *mecA*$_{LGA251}$ *MultiFP (5'-GAAAAAAGGCTTAGAACGCCTC-3') and mecA* $_{LGA251}$ *MultiRP (5'-GAAGATCTTTTCCGTTTTCAGC-3'),* described by Paterson *et al.* (33) were for used *mecC.* A 25µl reaction mixture containing 3µl of 10 x PCR buffer, 2µl deoxyribonucleotide triphosphates (DNTPs), 1µl of Taq polymerase (1unit/µl), 1µl of each primer and 2µl of the DNA extract was used. Amplification was carried out in an eppendorf master-cycler gradient using the following parameters: an initial denaturation at 94°C for 5 minutes and 30 cycles of 94°C for 1 minute, 59°C for 1 minute and 72°C for 1 minute. This was followed by a final extension of 72°C for 10 minutes. The PCR products were electrophoresed on a 1.5% agarose gel with addition of ethidium bromide and visualized using a UV light transilluminator. 100kb DNA ladder was used as DNA molecular weight standard. Positive results were inferred by detection of a 155-bp DNA band, which represented a part of the *mecA* gene. For *mec*C, a positive result was indicated by the presence of a 188 bp DNA fragment.

Table 1: *Staphylococcus aureus* isolated from animal and human sources

Sample Source	No. of Samples Collected	Frequency of *S. aureus* isolation (%)
Farm Personnel	25	16 (6.4%)
Cows	50	32 (12.8%)
Sheep	50	25 (10%)
Goats	50	24 (9.6%)
Pigs	50	32 (12.8%)
Farm Personnel	25	12 (4.8%)
Total	250	141 (56.4%)

RESULTS

The number of animals and farm personnel caring for the animals are presented on Table 1. The occurrence of *S. aureus* was 56.5% (113/200) and 56.0% (28/50) from the livestock and the personnel, respectively. The isolation rate was highest in cows and pigs followed by sheep. Table 2 describes the antibiotic susceptibility of the *S. aureus* isolates. A large proportion of the isolates was resistant to tetracycline

Table 2: Antibiotic susceptibility pattern of *Staphylococcus aureus* obtained in this study (n=141)

Antibiotics	*No. of Resistant Isolates (%)					Total No/ Resistance rate (%)
	Human (n=28)	Pigs (n=32)	Cows (n= 32)	Sheep (n=25)	Goat (n=24)	
Ciprofloxacin	19 (67.9)	16 (50)	11 (34.4)	7 (28)	3 (12.5)	56 (39.7)
Oxacillin	18 (64.2)	11 (34.3)	11(34.3)	6 (24)	9 (37.5)	55 (39.0)
Trimethoprim-Sulphamethoxazole	18 (64.2)	9 (28.1)	15 (46.8)	10 (40.0)	5 (20.8)	57 (40.4)
Cefuroxime	20 (71.4)	18 (56.2)	12 (37.5)	7(28.0)	12 (50.0)	69 (48.9)
Gentamycin	14 (50.0)	12 (37.5)	13 (40.6)	9 (36.0)	6 (25.0)	54 (38.2)
Ceftazidime	21 (75.0)	16 (50.0)	10 (31.2)	9 (36.0)	9 (37.5)	65 (46.0)
Fusidic acid	25 (89.2)	32 (100.0)	21 (65.6)	19 (76.0)	17 (70.8)	114 (80.8)
Cefoxitin	21 (75.0)	11 (34.3)	15 (46.8)	7 (28.0)	9 (37.5)	63 (44.6)
Erythromycin	24 (85.7)	18 (56.2)	13 (40.6)	25 (100.0)	11 (45.8)	91(64.5)
Ceftriaxone	21 (75.0)	12 (37.5)	16 (50.0)	9 (36.0)	6 (25.0)	64 (45.3)
Tetracycline	28 (100.0)	32 (100.0)	30 (98.7)	19 (76.0)	15 (62.5)	124 (87.9)
Amoxicillin/Clavulanate	19 (67.9)	17 (46.8)	15 (46.8)	8 (32.0)	5 (20.8)	64 (45.3)

* All intermediate resistance isolates were considered as resistant.

Table 3: Frequency of *mec*A and *mec*C genes among the *Staphylococcus aureus* isolates investigated

Isolate Source	No. of isolates	No. of isolates positive for:	
		*mec*A	*mec*C
Goats	6	0 (0%)	0
Sheep	6	0 (0%)	0
Cows	7	4 (8%)	0
Pigs	6	3 (6%)	0
Human	25	3 (6%)	0
Total	50	10 (20%)	0 (0%)

(87.9%: 124/141), fusidic acid (80.8%: 114/141) and more than 40% were resistant to cefuroxime, ceftazidime, cefoxitin, ceftriaxone, trimethoprim-sulphamethoxazole, amoxicillin – clavulanate and erythromycin. *mec*A was found in 10 of the 50 *S. aureus* isolates screened, giving an inclusive percentage of 20% (Table 3). Four *mec*A-positive strains were from cows and three were detected among pigs. Regarding the workers, *mec*A-positive *S. aureus* were identified in two individuals working on the pig farm while the third case of human-MRSA was found among the participants on the cattle site. While two isolates from goats (G10 and G17) showed susceptibility to all the antibiotics, multi-resistant methicillin susceptible *S. aureus* (MSSA) strains represented 87.9% (124/141) of the total *S. aureus* studied. The MSSA strains were grouped into thirteen categories of antibiotypes with various subtypes (Table 4). Resistance to tetracycline, fusidic acid, erythromycin and the beta-lactams was the most frequently observed. All *mec*A-positive *S. aureus* isolates demonstrated resistance to fusidic acid, oxacillin, ceftazidime, cefoxitin, ceftriaxone, cefuroxime, amoxicillin/clavulanate and tetracycline (Table 5). No *mec*C was detectable.

DISCUSSION

In the past few years, the characteristics of S. *aureus* has changed significantly due to the emergence of strains adapted to livestock (34) with differences in population and environment. This study therefore provides basic information about *S. aureus*-associated with livestock and the personnel tending the animals in the region under investigation. We observed an overall nasal carriage rate of 56.4% (141/250). In other part of Nigeria, the frequency of *S. aureus* from nasal swabs of livestock (cow, goat sheep and pig) has been seemingly at a low level (35). Also, the present culture positive rate was not in conformity with that reported in a Czech Republican study (36). However, the occurrence of *S. aureus* carriage among cattle, sheep and goat in Iran was higher (37) than that we observed herein. These differences could be attributed to locations, culture methods, host species and farm practices including the level of hygiene in the investigated farms. Nonetheless, our results suggest that the occcurence of *S. aureus* in livestock could be higher than previous estimates.

As observed in this study, the percentage of multidrug resistant *S. aureus* was unprecedented. This could lead to failure in treatment therapy and increased expenses for health care. The Federal Ministries of Agriculture, Environment and Health had anticipated the loss of effective antimicro-

Table 4: Multidrug Resistance Profiles of Methicillin Susceptible *Staphylococcus aureus* Isolates

Antibiotype	Susceptibility Pattern*												No. of Antibiotics	No. of Isolates by Source:					Total
	FOX	OXA	AMC	CRX	CTR	CAZ	TET	CIP	GEN	SXT	ERY	FUS		COW	GOAT	SHEEP	PIG	HUMAN	
A	R	R	R	R	R	R	R	R	R	R	R	R	12	2				4	6
B	R	R	R	R	R	R	R	R	R	S	R	R						3	
B1	R	R	R	R	R	R	R	R	R	R	S	R	11				1	2	
B2	R	R	R	R	R	R	R	S	R	R	R	R						3	13
B3	R	R	R	R	R	R	R	R	S	R	R	R				2		2	
C	R	R	R	R	R	R	R	R	S	S	R	R				2	3	2	
C1	R	R	R	R	R	R	R	S	R	S	R	R	10		1				12
C2	R	R	R	R	R	R	R	S	S	R	R	R			1		1	1	
C3	R	R	R	R	R	R	R	R	R	S	S	R					1		
D	R	R	R	R	R	R	R	R	S	S	S	R		2	1		2		
D1	R	R	R	R	R	R	R	S	S	R	S	R		2			2		
D2	R	R	R	R	R	R	R	R	S	S	R	S				1		1	
D3	R	R	R	R	R	R	R	S	S	R	R	S	9		1	1			18
D4	R	R	R	R	R	R	R	S	S	S	R	R					2		
D5	R	R	R	R	R	R	R	R	S	R	S	S		1				1	
D6	R	R	R	R	R	R	S	R	S	R	S	R			1				
E	R	R	R	R	R	R	R	S	S	S	S	R			1		1		
E1	R	R	R	R	R	R	R	S	S	S	R	S				1			
E2	R	S	S	R	R	S	R	R	R	R	S	R	8					1	7
E3	R	R	R	R	R	R	S	R	S	R	S	S		1					
E4	S	S	S	S	R	R	R	R	R	R	R	R				1			
E5	S	S	R	R	S	S	R	R	R	R	R	R		1					
F	R	R	R	R	R	R	R	S	S	S	S	S		1					
F1	R	S	S	S	R	S	R	R	S	R	R	R		1					
F2	R	S	R	R	R	R	R	S	S	S	R	S	7	1					5
F3	R	S	R	S	R	S	R	S	S	R	R	R						1	
F4	S	S	R	R	R	S	R	S	S	R	R	R		1					
G	R	S	S	S	S	S	R	S	R	R	R	R				1			
G1	R	S	S	R	S	R	R	R	S	S	R	S						1	
G2	S	S	S	R	S	S	R	S	R	R	R	R			1		1		
G3	S	S	S	S	S	R	R	S	R	R	R	R				1	1		
G4	S	S	S	S	S	R	R	R	R	R	R	S		2					
G5	S	S	S	S	S	R	R	R	R	R	S	R	6	1					13
G6	S	S	S	S	S	R	R	R	S	R	R	R						1	
G7	S	S	S	R	S	S	R	R	R	S	R	R			1				
G8	S	S	R	R	S	S	S	R	S	R	R	R				1			
G9	S	S	S	S	R	S	R	R	R	R	S	R					1		
H	R	R	S	S	S	S	R	S	S	R	S	R		1					
H1	S	S	S	S	S	R	R	R	S	S	R	R					1		
H2	S	S	S	S	S	S	R	S	R	R	R	R						1	
H3	S	S	S	S	S	S	R	R	S	R	R	R					1		
H4	S	S	S	S	S	R	R	S	R	S	R	R		1					
H5	S	S	S	S	S	R	R	S	S	R	R	R						1	
H6	S	S	S	R	S	S	R	S	R	S	R	R					1		
H7	S	S	S	R	S	S	R	R	S	S	R	R	5				1		15
H8	S	S	S	R	S	S	R	R	R	S	S	R					1		
H9	S	R	S	S	R	S	R	S	S	R	S	R			1				
H10	S	S	R	S	S	S	R	S	R	R	S	R					1		
H11	S	S	R	S	S	S	R	S	R	S	R	R					1		
H12	S	S	S	S	R	S	R	S	R	S	R	R				1			
H13	S	S	R	S	S	R	R	R	S	S	S	R					1		
H14	S	S	R	S	R	S	R	S	S	S	R	R		1					
I	R	S	S	S	S	R	R	S	S	S	S	R			1				
I1	R	S	R	S	S	S	R	S	S	S	S	R		1					
I2	R	S	S	S	S	S	R	R	S	R	S	S		1					
I3	R	S	S	S	S	S	R	S	R	S	S	R		1					
I4	R	R	S	S	S	R	R	S	S	S	S	S			1				
I5	R	R	S	S	S	S	R	S	S	S	R	S			1				
I6	S	S	S	R	S	S	R	S	S	S	R	R					2		
I7	S	S	S	S	S	S	R	S	R	S	R	R				2	1		
I8	S	S	S	S	S	S	R	S	R	R	R	S		1		1			
I9	S	S	S	S	S	S	S	S	R	R	R	R	4			1			24
I10	S	S	S	S	S	R	R	S	S	S	R	R					1		
I11	S	S	S	S	S	S	R	R	S	S	R	R						1	
I12	S	S	S	R	S	R	S	S	S	S	R	R			1				
I13	S	S	S	R	S	S	S	S	R	S	R	R		1					
I14	S	S	R	S	R	S	R	S	S	S	R	S		1					
I15	S	S	R	S	R	S	R	S	S	S	S	R		1					
I16	S	S	R	S	S	S	R	S	S	R	S	R		1					
I17	S	S	R	S	S	S	R	S	R	S	S	R					1		
I18	S	S	R	S	S	S	R	R	S	S	S	R					1		
I19	S	S	R	S	S	R	S	S	S	S	R	R				1			
J	S	S	S	S	S	S	R	S	S	S	R	R			1	3		1	
J1	S	S	S	R	S	S	R	S	S	S	S	R		1	1				
J2	S	S	S	S	R	S	R	S	S	S	S	R		1				1	
J3	S	S	S	S	S	S	S	S	R	S	R	R			1	1			
J4	S	S	S	S	S	S	R	R	S	S	S	R					1		
J5	S	S	S	S	S	S	R	S	R	S	S	R			1				
J6	S	S	S	S	S	S	R	S	R	S	R	S		1					
J7	S	S	S	S	S	S	R	S	S	R	S	R	3				1		21
J8	S	S	S	S	S	S	R	S	S	R	R	S				1			
J9	S	S	S	R	S	S	R	S	S	S	R	S			1				
J10	S	S	S	S	S	R	S	S	S	S	R	R			1				
J11	S	R	S	S	S	S	R	S	S	S	S	R		1					
J12	S	S	S	R	S	S	S	S	R	S	S	R			1				
J13	S	R	S	S	S	S	R	S	R	S	S	S		1					
K	S	S	S	S	S	S	R	S	S	S	S	R				1			
K1	S	S	S	S	S	S	S	S	S	S	R	R	2			1			3
K2	S	S	S	S	R	S	S	S	S	S	R	S				1			
L	S	S	S	S	S	S	S	S	S	S	S	R	1		1				2
L1	S	S	S	R	S	S	S	S	S	S	S	S			1				
M	S	S	S	S	S	S	S	S	S	S	S	S	0		2				2

* See the text for abbreviation.

Table 5: Antibiotic susceptibility profiles of *mec*A-positive *S. aureus* isolates

Isolate Code	Source	FOX	OXA	AMC	CRX	CTR	CAZ	TET	CIP	GEN	SXT	ERY	FUS	Antibiotype
C2	Cow	R	R	R	R	R	R	R	R	R	R	R	R	A
C4	Cow	R	R	R	R	R	R	R	R	R	R	R	R	A
C9	Cow	R	R	R	R	R	R	R	R	S	R	S	S	D5
C16	Cow	R	R	R	R	R	R	R	R	S	S	S	R	D
P3	Pig	R	R	R	R	R	R	R	S	R	S	R	R	C1
P15	Pig	R	R	R	R	R	R	R	R	S	S	S	R	D
P20	Pig	R	R	R	R	R	R	R	S	S	R	S	R	D1
1H10	Cattle Farm Personnel	R	R	R	R	R	R	R	S	R	R	R	R	B2
2H5	Pig Farm Personnel	R	R	R	R	R	R	R	S	S	R	R	R	C2
2H9	Pig Farm Personnel	R	R	R	R	R	R	R	R	S	S	R	S	D2

R=Resistant; S= Susceptible.

bials due to resistance in Nigeria (23). Antimicrobial use and misuse are driven by patients, farmers and the general populace who demand antimicrobials for real or presumed infections and procure them from unsanctioned sources even when they are not prescribed (23, 38). The present results are comparable with those of Gulani *et al*. (39), who observed high resistance to erythromycin, tetracycline and cefoxitin among strains of *S. aureus* from ruminants (cattle, sheep, goats) and animal handlers in Maiduguri, Northern Nigeria. Their study however found that the frequency of resistance of the isolates to ciprofloxacin, sulphamethoxazole, oxacillin and gentamycin was low. The resistant phenotypes of our *S. aureus* isolates were such that resistance to tetracycline > fusidic acid > erythromycin > cefoxitin > amoxicillin/clavulanate, ceftriaxone, ceftazidime and ceftazidime > cefuroxime > trimethoprim - sulphamethoxazole > ciprofloxacin > oxacillin > gentamycin. It could therefore be presumed that antimicrobial usage across the two farms investigated was very high.

While studies (40) of clinical *S. aureus* isolates in Nigeria have described low fusidic acid resistance (21, 41), surprisingly, about 80% of *S. aureus* strains examined in this study exhibited resistance to fusidic acid. Unfortunately, the extent of fusidic acid prescription and accessibility in Nigeria is uncertain and this makes it impossible to provide the justification for its use in our farms. Consequently, this finding may have important clinical implications since the antibiotic has been suggested in the treatment of staphylococcal infections in humans (42). Moreover, the recovery of fusidic acid- resistant strains in this study calls for vigilance as it may be an integral part of staphylococcal epidemiology. Nevertheless, the occurrence of fusidic acid resistant strains in the present study was comparable to data published on MRSA isolates in an Algiers Hospital (43). The *mec*-PCR analysis of fifty isolates selected revealed that 20% harbored *mec*A gene. Elsewhere, studies carried out on healthy livestock also showed a lower prevalence of MRSA. For example, Rahimi *et al*. (37) identified one *mec*A-MRSA (1/26; 3.84%) from sheep. Likewise, a survey in Tunisia detected five MRSA strains amongst 163 samples from healthy sheep (44). In contrast, high MRSA colonization rates of 44% and 12.6% have been documented among pigs – *S. aureus* from a large scale study in Belgium (45) and South Africa (46) respectively. The proportion of MSSA obtained in this study was consistent with the findings of other studies (25, 39).

The occurrence of nasal-MRSA among workers in the present study was 6% (3/25). A few findings in this country have shown that human MRSA colonization rate is modest (41). Variation of MRSA colonization in contact persons have also been recognized by authors in other countries (47, 48). However, the comparative analysis of our results with others seems challenging since different variables were considered. Further experiments are warranted to determine the genetically relatedness of the isolates. Notwithstanding, the results presented here show that the occurrence of nasal-MRSA among the farm workers may represent a potential risk for successive transmission into the community. Furthermore, our findings confirmed that pig workers have higher potential of MRSA colonization (49). Therefore, efforts need to be directed at recognizing possible reservoirs in order to reduce the spread of *mec*A-MRSA. *mec*A gene homologue

(*mec*C) – *S. aureus* which is assumed to represent important strains which can cause disease in livestock and humans. Using the protocol described by Paterson *et al*. (33), our results showed that none of the isolates harbored the *mec*C gene. It thus appears that *mec*C-carrying *S. aureus* isolates is rare in this setting. A limitation of this study is the fact that *mec* screening was performed only on the isolates with β-lactam and cephalosporins resistance and thus we may have underestimated the occurrence of MRSA carriage on the two farms.

Nevertheless, our findings indicated a high occurrence of *S. aureus* and recognized the presence of *mec*A-MRSA with phenotypic multidrug resistance traits. The heterogeneity of the resistance profiles of the MSSA underlines a possible existence of antibiotic selective pressure in the farms. Therefore, these results serve as a template for surveillance studies to elucidate the channel of dissemination of antibiotic resistance and reservoirs of MRSA in Nigeria.

CONFLICT OF INTEREST STATEMENT

We did not receive funds for this work from any agencies and there is no conflict of interest to declare.

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