



Prevalence and Antibiotic Susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Bovine Mastitis in Settled Fulani Herds in Kaduna State

Umaru, G.A.¹; Kwaga, J.K.P.²; Bello, M.²; Raji, M.A.²; Maitala, Y.S.²; Junaidu, K.²

¹Department of Animal Health, College of Agriculture, P.M.B., 1025, Jalingo, Taraba State.

²Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, P. M. B. 1045, Samaru, Zaria- 810271. Corresponding author email: drghaliumaru@yahoo.com, Tel No; +23486897535

SUMMARY

A study was conducted to determine the prevalence and antibiotic resistance of methicillin resistant *Staphylococcus aureus* (MRSA) from bovine mastitis in settled Fulani herds in Kaduna state. Three hundred and sixty milk samples randomly collected from selected herds in Kaduna South, Igabi, Lere, Sabon-Gari, Giwa and Zaria were examined. The prevalence of mastitis at cow level was 26.9%, out of which 3.1% and 23.9% were clinical and subclinical mastitis, respectively. The prevalence of both *S. aureus* and MRSA were 15.3% and 7.8 % respectively. Significant proportion (42.9%) of the MRSA were isolated from cases of mastitis ($P < 0.05$). Very high percentages of MRSA were resistance to penicillin (100.0%), amoxicillin (89.3%), ampicillin (89.3%), tetracycline (85.7%) and erythromycin (71.1%). The multiple drug resistance indices of the MRSA strains revealed that all the MRSA strains were resistant to 5 or more antibiotics tested; 6 (21.4%) strains were resistant to 6 antibiotics, 6 (21.4%) were resistant to 7 antibiotics, 8 (28.6%) were resistant 8 antibiotics and 4 (14.3%) were resistant to 9 antibiotics, while 19 (67.9%) exhibited the extensive drug classification pattern. The MIC values of the antibiotics showed that all (100%) have values greater than 256 $\mu\text{g/mL}$ against oxacillin while 15 (53.6%) have values greater than 256 $\mu\text{g/mL}$ against vancomycin, respectively. Nineteen (67.9%) of the MRSA isolates tested for PBP2a gave strongly positive (3+ to 4+) reactions in the Latex Agglutination Test, 5 (1.9%) were weakly positive (1+) in the Latex Agglutination Test while 4 (14.3%) were negative. The occurrence of MRSA in bovine mastitis is of public health concern due to difficulty in treatment of staphylococcal diseases and possible transmission of resistant pathogens to humans. Therefore, continuous monitoring and surveillance of antibiotic resistance pathogens, good husbandry practices, culling of infected cows and pasteurization of milk to eliminate MRSA and other pathogens are recommended

Key words: Prevalence, antibiotic susceptibility, MRSA, Bovine mastitis, cow milk, multi drug resistance, extensive drug resistance (XDR), minimum inhibitor concentration, Penicillin Binding Protein 2a

INTRODUCTION

S. aureus is one of the most common and economically important pathogens associated with intramammary infections in dairy herds (Cabral *et al.*, 2004), and has been responsible for 30-40% mastitis cases (Pereira *et al.*, 2009; Haram *et al.*, 2013). Therefore the micro-organism can contaminate milk from udder with clinical or subclinical mastitis or from the environment during manipulation and processing (Reinoso *et al.*, 2008; Mirzaei *et al.*, 2011). It can easily grow and multiply in milk and related products and may produce enterotoxins (Rahimi *et al.*, 2008; Intrakamhaeang *et al.*, 2012).

Treatment of *S. aureus* infections after penicillin resistance was with the semi-synthetic penicillin drugs such as methicillin (Livermore, 2000). However, methicillin-resistant *S. aureus* (MRSA) was discovered in 1960, and were identified as a major cause of nosocomial infections (Jevons, 1961). Since then transmission of health-care associated MRSA (HA-MRSA) to community was frequently reported. Hence, MRSA was increasingly found in the community (community-associated-methicillin-resistant *S. aureus*) (CA-MRSA) resulting in severe skin and soft tissues infections and necrotizing pneumonia. Similarly, livestock-associated (LA) MRSA genotypically classified under clonal complex 398 (CC398) has been detected in pigs and swine farmers in the Netherlands and other countries (Khanna *et al.*, 2008; Denis *et al.*, 2009) and is known to cause infections in humans and animals (Lewis *et al.*, 2008. Declerog *et al.*, 2008).

MRSA was first reported from bovine sources in 1975, and has occasionally been reported since then in different parts of the world including Nigeria (Mirzaei *et al.*, 2011; Kreauskon *et al.*, 2012; Haram *et al.*, 2012; Erdem and Tükyilmaz, 2013; Paterson *et al.*, 2013; Unnerstad *et al.*, 2013; Umaru *et al.*, 2013). The presence of MRSA in bovine milk and dairy environment poses potential risk to farm workers (Erdem and Tükyilmaz, 2013), veterinarians (Lee, 2003), and farm animals that

are exposed to contaminated cattle (Intrakamhaeang *et al.*, 2012) with cow to human transmission having been established (Erdem and Tükyilmaz, 2013). Therefore, this study was carried out to determine the occurrence and antibiotic susceptibility of MRSA in fresh cow milk and its association with mastitis in settled Fulani herds in Kaduna State, Nigeria.

MATERIAL AND METHODS

Study Areas

This research was conducted in Kaduna State, Nigeria. Kaduna State is located at the centre of Northern Nigeria with the coordinate's 10°31'N and 7°26'E; 10°52'N and 7°43'E. The state shares boundaries with Niger state to the west, Zamfara, Katsina and Kano states to the north, Bauchi and Plateau states to the east and FCT, Abuja and Nasarawa states to the south (Fletcher and Dan, 1996). Kaduna State is located in the savannah zone, which is characterized by short wet season and long dry season. Agriculture is therefore the main stay of the economy. Another major occupation of the people is animal rearing, namely cattle, sheep, goats and pigs. (Kaduna State, 2010). The cattle population of the state as at 1990 was 1, 006, 634 with an annual estimated increase of 1.5% (RIMS Report, 1990).

Sample Size and Sample Collection

The number of sample was determined using the Fisher's formula (Kasiulevičius *et al.*, 2006)

$$n = \frac{Z^2 P (1-P)}{e^2} = \frac{(1.96)^2 \times 0.356 \times (1-0.356)}{(0.05)^2}$$

Where:

n = Desired sample size for the study

Z = Desired confidence level (1.96 for 95% confidence level)

P = Prevalence of MRSA (35.6%, Suleiman *et al.*, 2012)

1 - p = Proportion of cows without MRSA (1-0.048)

e² = Desired level of precision (5% or 0.05)

Therefore n = 352

Using a confidence level of 95%, an expected prevalence of 35.6% (Suleiman *et al.*, 2012) of

MRSA from bovine mastitic, 352 milk samples would have to be collected. However, the sample size was increased to 360 to increase precision. Therefore, 360 milk samples were aseptically collected from randomly selected herds and also randomly selected cows in Kaduna South, Igabi, Lere, Sabon-Gari, Giwa and Zaria according to a standard protocol. The milk samples were placed on ice and transported to the Bacterial Zoonoses laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for standard laboratory processing and isolation.

Clinical and subclinical mastitis test

Clinical mastitis was assessed by observing the udder visually and by palpation for visible trauma, tick infestation, pain, warmth and swelling of the super mammary gland (Mekibib *et al.*, 2010). Abnormality in milk characteristics like blood tinged milk, watery secretions, clots and pus were checked (Dinwell *et al.* (2003). Cows that did not have clinical mastitis were subjected to further examination for subclinical mastitis by using California Mastitis Test (CMT) as described by Dinwell *et al.* (2003). Foremilk from each quarter was milked into cups of four-cup plastic paddle. The paddle was tilted to equalize milk quantities in the cups at 2.5 ml each. Equal volume of the CMT reagent (Kruuse, Denmark) was added to each cup. The paddle was rotated to mix thoroughly. Changes in colour and gel formation were observed within 10-15 seconds after mixing and then scored depending upon the amount of gel formation as follows:

No reaction= Negative

Appearance of streaks visible during rotation of the plate= Trace

Distinct thickening during rotation, but no gel= 1+

Slight formation of gel which follows the rotating plate very slowly= 2+

Solid formation of gel that adheres to the base of the plate= 3+

Isolation and characterization of *Staphylococcus aureus*

S. aureus was isolated by culturing the milk

samples on Baird Parker medium supplemented with egg yolk and potassium tellurite (Tamagnini *et al.*, 2006) followed by characterization using colony morphology, gram staining, catalase, coagulase, motility, DNase, sugar fermentation, pigmentation and haemolysis tests (Quinn *et al.*, 1994; Japoni *et al.*, 2004; Normano, 2005; Tamagnini *et al.*, 2006); and commercial identification system, Microbact™ 12S Staphylococcal Identification System (Oxoid, Basingstoke, UK)

Isolation and Characterization of Methicillin Resistant *Staphylococcus aureus*

All coagulase-positive *S. aureus* were screened for Oxacillin resistance using Oxacillin Resistance Screening Basal (ORSAB) medium (Oxoid, Basingstoke, United Kingdom). Growth of colonies that showed blue coloration were considered as potential MRSA, while those with no growth or colonies with colours other than blue were considered as negative. The potential MRSA were further confirmed by antibiotic susceptibility test using 1 µg oxacillin and 30 µg cefoxitin discs by disc diffusion method, and then confirmed for penicillin binding protein 2a (PBP2a) with the PBP2' Latex Agglutination Test (Oxoid Basingstoke, United Kingdom) (Paterson *et al.*, 2013).

Antimicrobial Susceptibility Testing

All confirmed MRSA were tested for resistance to a panel of 12 antibiotics using the disc diffusion method outlined by the Clinical Laboratory Standard Institute (CLSI, 2014), and minimum Inhibitory Concentration (MIC) values for oxacillin and vancomycin were determined. All isolates with MIC >4µg/mL and >16 µg/mL were considered oxacillin and vancomycin resistant, respectively (CLSI, 2014).

The antibiotics and their concentrations are: amoxicillin (30 µg), ampicillin (30 µg), chloramphenicol (12 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), penicillin (10 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim (5 µg) and vancomycin (30 µg)

Data Analysis

All data were statistically analyzed using SPSS package (Version 16.0). Probability values of statistical significance among prevalence of mastitis, *S. aureus* and MRSA in milk from different locations were determined using Chi-square and Fisher's exact test at 5% level of confidence. A P value of $P < 0.05$ was considered as significant.

Antimicrobial susceptibility results were analyzed for *S. aureus* and MRSA to observe trends in resistance for each tested antibiotic. Multiple antimicrobial resistance index (MARI) was determined for each isolate using the formula: $MARI = x/y$, where, x is the number of antibiotic to which an isolate is resistant to and y is the total number of antibiotics tested. Association between mastitis, *S. aureus*, MRSA and antimicrobial susceptibility were also assessed.

RESULTS

The prevalence of mastitis at cow level was 26.9%, out of which 3.1% and 23.9% were clinical and subclinical mastitis respectively (Table 1). The prevalence of both *S. aureus* and MRSA obtained was 15.3% and 7.8%, respectively. There was significant difference ($P < 0.05$) in the occurrence of *S. aureus* and MRSA in the study area. The prevalence of both *S. aureus* and MRSA obtained was 15.3% (55/360) and 7.8% (28/360) respectively (TABLE 1).

Occurrence of MRSA with respect to mastitis showed that 12 (42.9%) of the 28 MRSA were associated with mastitis ($P < 0.05$) (TABLE 2). Four (4) of the MRSA from Kaduna South and Lere were associated with mastitis, 2 isolates were associated with mastitis in Zaria and one each from Giwa and Igabi respectively. None of the 4 MRSA isolates from Sabon Gari was associated with mastitis (TABLE 2).

TABLE 1: Prevalence of clinical and sub clinical mastitis in cows and fresh milk samples in settled Fulani herds in Kaduna State, Nigeria

Location	No. of samples examined	No. (%) of Clinical mastitis	No. (%) of Subclinical mastitis	Total mastitis
Giwa	60	2 (3.3)	13 (21.7)	15 (25.0)
Igabi	60	3 (5.0)	18 (30.0)	21 (35.0)
Kaduna South	60	2 (3.3)	16 (26.7.0)	18(30.0)
Lere	60	4 (6.7)	15 (25.0)	19 (31.7)
Sabon-Gari	60	0 (0.0)	8 (13.3)	8 13.3)
Zaria	60	0 (0.0)	16 (26.7)	16 (26.7)
Total	360	11 (3.1)	86 (23.9)	97 (26.9)

$$\chi^2 = 41.722, df = 15, P = 0.000$$

TABLE 2: Prevalence of MRSA in relation to mastitis in cow milk in settled Fulani herds in Kaduna State, Nigeria

Location	No. of milk examined	No. (%) mastitis	No. (%) positive for <i>S. aureus</i>	No. (%) MRSA	No. (%) of MRSA with mastitis
Giwa	60	15 (25.0)	3 (5.0)	3 (5.0)	1 (33.3)
Kaduna South	60	21 (35.0)	11 (18.3)	8 (13.3)	4 (50.0)
Igabi	60	18 (30.0)	7 (11.7)	1 (1.7)	1 (100.0)
Lere	60	19 (31.7)	16 (26.7)	8 (13.3)	4 (50.0)
Sabon-Gari	60	8 13.3)	10 (16.7)	4 (6.7)	0 (0.0)
Zaria	60	16 (26.7)	8 (13.3)	4 (6.7)	2 (50.0)
Total	360	97 (26.9)	55 (15.3)	28 (7.8)	12 (42.9)

$$\chi^2 = 41.722, df = 15, P = 0.000$$

The 28 MRSA isolates recorded high resistance against penicillin 28 (100.0%), amoxicillin 23 (89.3%), ampicillin 23 (89.3%), tetracycline 24 (85.7%) and erythromycin 20 (71.1%) (Table 3). The multiple drug resistance indices of the MRSA strains revealed that all the 28 MRSA strains were resistant to 5 or more antibiotics tested; 6 (21.4%) strains were resistant to 6 antibiotics, 6 (21.4%) were resistant to 7 antibiotics, 8 (28.6%) were resistant to 8 antibiotics and 4 (14.3%) were resistant to 9 antibiotics (Table 4). Also, significant number ($P < 0.05$) of the isolates (92.9%) exhibited different resistant patterns from each other with only four (4) exhibiting

(100%) have values greater than 256 $\mu\text{g/mL}$ against oxacillin and 15 (53.6%) have values greater than 256 $\mu\text{g/mL}$ against vancomycin respectively. Thirteen (46.4%) had MIC values $< 4 \mu\text{g/mL}$ against vancomycin (TABLE 4).

The results further showed that 19 (67.9%) of the 28 MRSA isolates tested for PBP2a Latex Agglutination Test gave strongly positive (3+ to 4+) reactions in the Latex Agglutination Test, 5 (1.9%) were weakly positive (1+) in the Latex Agglutination Test while 4 (14.3%) were negative (TABLE 4).

DISCUSSION

The prevalence of MRSA (7.8%) was very high when compared to 0% in US bulk tank milk (Virgin *et al.*, 2009), 1.4% in raw milk in Switzerland (Huber *et al.*, 2010), 4% in Bulk Tank Milk from Minnesota Dairy Farms (Haran *et al.*, 2012), 2.15% in bovine bulk tank milk in Great Britain (Paterson *et al.*, 2013) and 4.8% from our previous study in Zaria and Kaduna respectively (Umaru *et al.*, 2013). The result is however lower than 29.3% in raw milk in Chonju, Republic of Korea (Lee, 2003), 14.7% in raw goat's milk in Czech Republic (Strastkova *et al.*, 2009) and 11.25% in Dairy

TABLE 3: Antibiotic resistance and susceptibility of MRSA isolated from cow milk in settled Fulani herds, Kaduna State, Nigeria (N=28).

Antibiotic	No. (%) susceptible	No. (%) intermedia te resistant	No. (%) resistant
Amoxicillin	3 (10.7)	0 (0.0)	25 (89.3)
Ampicillin	3 (10.7)	0 (0.0)	25 (89.3)
Chloramphenicol	17 (60.7)	3 (10.7)	8 (28.6)
Ciprofloxacin	26 (92.9)	2 (7.1)	0 (0.0)
Erythromycin	7 (25.0)	1 (3.6)	20 (71.4)
Gentamicin	16 (57.1)	1 (3.6)	11 (39.3)
Nalidixic acid	13 (46.4)	0 (0.0)	15 (53.6)
Penicillin	0 (0.0)	0 (0.0)	28 (100.0)
Streptomycin	16 (57.1)	0 (0.0)	12 (42.9)
Tetracycline	4 (14.3)	0 (0.0)	24 (85.7)
Trimethoprim	18 (64.3)	3 (10.7)	7 (25.0)
Vancomycin	13 (46.4)	0 (0.0)	15 (53.6)

common patterns of multiple resistances.

The 2 patterns encountered were AML,AMP,C,CN,E,NA,P,S,TE exhibited by isolates K23 and K42 and AML,AMP,E,P,TE,VA,W exhibited by isolates K3 and S26 (Table 4). Nineteen (67.9%) exhibited the extensive drug classification pattern (Table 4). The MIC values of the 28 MRSA isolates showed that all

Farms of Pokhara, Nepal (Joshi *et al.*, 2014). Similarly, the present result is lower than the 25% obtained by Omshaba *et al.* (2018) in raw milk and soft cheese (*wara*) sold in Abeokuta, Nigeria. The difference in prevalence is statistically significant, although the reasons for this are not obvious, especially as MRSA has been isolated from milk and other animals in Nigeria including

humans (Umaru *et al.*, 2013, Bala *et al.*, 2016).

TABLE 4: Multiple antibiotic resistance profile, antibiotic classification and multiple resistances Index of MRSA isolated from cow milk in Kaduna State, Nigeria (N=28)

MRSA	Resistance pattern	NART	ARC	MARI	PBP2a		MIC	
							($\mu\text{g/mL}$)	
							OX	VA
K3	AML,AMP,E,P,TE,VA,W	6		XDR	0.5	Negative	256	256
K14	AML,AMP,C,CIP,CN,R,P,S,TE	9		XDR	0.8	Weak positive	256	<4
K20	AML,AMP,E,P,S,TE,VA	7		XDR	0.6	Positive	256	256
K23	AML,AMP,C,CN,E,NA,P,S,TE	9		XDR	0.8	Positive	256	25
K32	C,CN,E,NA,P,S,TE	7		MDR	0.6	Positive	256	<4
K34	AML,C,E,P,TE,VA	6		MDR	0.5	Positive	256	256
K42	AML,AMP,C,CN,E,NA,P,S,TE	9		XDR	0.8	Positive	256	<4
K57	AML,AMP,C,E,P,TE,VA	7		XDR	0.6	Positive	256	256
S1	AML,AMP,C,CN,E,P,TE,VA	8		XDR	0.7	Positive	256	256
S2	AML,AMP,C,CN,E,NA,P,TE	8		XDR	0.7	Positive	256	<4
S5	C,CN,P,S,R,VA	6		MDR	0.5	Weak positive	256	256
S6	AML,AMP,C,CN,NA,P,S,TE	8		XDR	0.7	Positive	256	<4
S20	AML,AMP,CIP,E,NA,P,TE	7		XDR	0.6	Weak positive	256	<4
S26	AML,AMP,E,P,TE,VA,W	7		XDR	0.6	Positive	256	256
S42	AML,AMP,E,P,W	5		MDR	0.4	Weak positive	256	<4
S60	AML,AMP,E,NA,P,TE	6		MDR	0.5	Positive	256	<4
I59	AML,AMP,C,E,P,TE,VA,W	8		XDR	0.7	Positive	256	256
SB24	AML,AMP,CN,E,NA,P,S,TE	8		XDR	0.7	Positive	256	<4
SB34	AML,AMP,CN,NA,P,S,TE	7		XDR	0.6	Positive	256	<4
SB51	AML,AMP,E,NA,P,TE,VA,W	8		XDR	0.7	Positive	256	256
SB60	AML,E,P,TE,VA,W	6		MDR	0.5	Positive	256	<4
G3	AML,AMP,NA,P,S	5		MDR	0.4	Weak positive	256	<4
G23	AML,AMP,NA,P,TE	5		MDR	0.4	Positive	256	<4
G25	AML,AMP,P,VA,W	5		MDR	0.4	Positive	256	256
Z10	AML,AMP,CN,E,P,VA	6		MDR	0.5	Negative	256	256
Z39	AML,AMP,E,P,S,TE,VA,W	8		XDR	0.7	Negative	256	256
Z47	AML,AMP,C,NA,P,TE,VA,W	8		XDR	0.7	Positive	256	256
Z49	AML,AMP,CN,E,NA,P,TE,VA,W	9		XDR	0.8	Negative	256	256

Possible explanation for the significant occurrence of MRSA in milk in this parts of Nigeria may be due to unrestricted and uncontrolled use of antibiotics in animal farming, an unsatisfactory health status of cattle herds compared to many countries in Europe and U.S.A., and also the fact that greater percentage of cattle herds are extensively managed, which exposed them to contaminated environment. Also, the two study areas have the largest hospitals in Kaduna state, and MRSA of hospital origin and being nosocomial pathogens may be transferred from the hospitals to the environment where animals can then be infected. The detection MRSA strains in cases of bovine mastitis may be the first of such report in the study area, This

finding is in agreement or coincides with several other studies. For example Türkyilmaz *et al.* (2010) reported that 16 of the 93 *S. aureus* isolated from bovine mastitic milk were resistant to methicillin. Also in a study by Itrakamhaeng *et al.* (2012), it was discovered that 4 of the 375 *S. aureus* from bovine mastitis cases were methicillin resistant. Similar report was observed in Nigeria by Suleiman *et al.* (2012) who revealed that of the 103 *S. aureus* isolated from mastitic milk, 26 (35.6%) were resistant to oxacillin. The present study also concur with the report of Helal *et al.*, (2015) who confirmed 7 (77.8%) MRSA isolates out of total 9 *S. aureus* isolates from cow mastitic milk, while, only 2 (22.2%) *S. aureus* isolates showed non-MRSA. *S. aureus* is one of the most important bacterial

pathogens in bovine mastitis, a disease that causes significant economical losses in the milk industry; thus, *S. aureus* in general and MRSA in particular have been the focus of several studies in dairy cattle. The occurrence and spread of MRSA in milk and dairy and other animals is of serious public health concern because of possible transmission between cows and humans (Itrakamhaeng *et al.*, 2012). The reports of Juhász-Kaszanyitzky *et al.* (2007), Erdem and Türkyilmaz (2013) and Helal *et al.* (2015) showed that bovine and human MRSA strains are epidemiologically related and indistinguishable, which indicates transmission from either bovine to human or human to bovine. In addition, MRSA may contaminate foods and represents a source of MRSA infection and intoxication.

The significant number of MRSA isolated from cow milk in these areas was probably caused by excessive therapeutic use of antibiotics. *S. aureus* can adapt rapidly to the selective pressure of antibiotics and becomes methicillin resistant by acquisition of *mec A* gene. This gene encodes a Penicillin Binding Protein (PBP2a) with low affinity for β -lactams (van Duijkeren *et al.*, 2004; Weese, 2005).

The resistance patterns of the MRSA isolates showed high resistance against penicillin 28 (100.0%), amoxicillin 23 (89.3%), ampicillin 23 (89.3%), tetracycline 24 (85.7%) and erythromycin 20 (71.1%) (Table 3). This report is similar to our previous study in the same area in which high resistance was recorded against Penicillin (100%), Tetracycline (55.5%), Oxacillin (55.6%), Vancomycin (44.4%), Amoxicillin (38.9%) and Erythromycin (27.8%). Türkyilmaz *et al.* (2010) also showed the MRSA strains to be multi-drug resistant with susceptibility rates to antimicrobials to be 0%, 0%, 0%, 0%, 6.25%, 16.25% and 56.25% for erythromycin, clindamycin, chloramphenicol, gentamicin, tetracycline, ciprofloxacin and vancomycin, respectively.

The multiple resistant pattern of the MRSA encountered showed that 26 (92.9%) exhibited different resistant pattern to each other, while only four (4) of the isolates exhibited common patterns of multiple resistance. The 2 patterns encountered

were AML,AMP,C,CN,E,NA,P,S,TE exhibited by isolates K23 and K42 and AML,AMP,E,P,TE,VA,W exhibited by isolates K3 and S26 (Table 4).

The multiple antibiotics resistance was classified as Extensive Drug Resistance (XDR), which is defined as non-susceptible of an isolate to ≥ 1 agent in all but ≤ 2 categories and Pan Drug Resistance (PDR) which is the non-susceptible of an isolate to all antimicrobial agents listed (Magiorakos *et al.*, 2012). The results showed that 19 (67.9%) of the MRSA exhibited the extensive drug classification pattern (TABLE 4). This report is similar to our previous study in the same area in which high resistance was recorded against penicillin (100%), tetracycline (55.5%), oxacillin (55.6%), vancomycin (44.4%), amoxicillin (38.9%) and erythromycin (27.8%). Türkyilmaz *et al.* (2010) also showed the MRSA strains to be multi-drug resistant with susceptibility rates to antimicrobials to be 0%, 0%, 0%, 0%, 6.25%, 16.25% and 56.25% for erythromycin, clindamycin, chloramphenicol, gentamicin, tetracycline, ciprofloxacin and vancomycin, respectively. Omoshaba *et al.* (2018) showed that apart from resistance to the beta-lactam antibiotics (ampicillin, ceftazidime, augmentin, cefoxitin, oxacillin), the MRSA isolates obtained from their study also demonstrated to varying degrees, resistance to other antimicrobials including aminoglycosides (streptomycin, gentamicin), tetracyclines (tetracycline and doxycycline), colistin and sulphomethoxazole. They concluded that selection pressure due to indiscriminate antimicrobial usage in food-producing animals is a major factor contributing to the emergence and dissemination of antimicrobial resistant bacterial strains Omoshaba *et al.*, 2018). Magiorakos *et al.* (2012) showed that emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria.

This indicates that all the 28 MRSA isolates and

46.4% were oxacillin and vancomycin resistant respectively. This trend was also reported by Aklilu *et al.* (2013) who showed that the MIC values of the MRSA isolates ranged from 1.5 µg/mL to more than 256 µg/mL with 5 isolates having MIC values <4 µg/mL, while 4 showed MIC ≥ 256 µg/mL. Also the Penicillin Binding Protein 2a (PBP2a) test of the 28 MRSA isolates showed that 19 (67.9%) gave strongly positive (3+ to 4+) reactions in the Latex Agglutination Test, 5 (1.9%) were weakly positive (1+) in the Latex Agglutination Test, while 4 (14.3%) were negative (TABLE 4). This is in agreement with Bressler *et al.* (2005) who tested 388 isolates with both the PBP2a LAT and the MicroScan PC20 panel for resistance to oxacillin and found 249 (64%) to be resistant to oxacillin based on the MIC results. All of the isolates for which the oxacillin MICs were >2 µg/mL gave strongly positive (3+ to 4+) reactions in the LAT. Three of the 139 isolates that were susceptible to oxacillin were also strongly positive, and 1 was repeatedly weakly positive (1+) in the LAT. This indicated that the isolates were MRSA. Omoshaba *et al.* (2018) obtained 50 (25%) methicillin resistant *Staphylococcus aureus* (MRSA) isolates from the 200 samples examined based on their resistance to oxacillin and cefoxitin as well as the presence of penicillin-binding protein 2a (PBP2a) as the case of the present study.

CONCLUSION AND RECOMMENDATIONS

Results obtained from this study revealed that MRSA were one of the causes of bovine mastitis, and has spread between different dairy locations within settled Fulani herds in Kaduna State. This is of public health concern due to difficulty in treatment of staphylococcal diseases and possible transmission of resistant pathogens to humans through consumption of contaminated milk. Therefore, continuous monitoring and surveillance of antibiotic resistance pathogens, proper hygienic measures during milking, good husbandry practices and culling of infected cows are recommended. Also, all milk for human consumption should

be pasteurized to eliminate MRSA and other antibiotic resistance pathogens.

ACKNOWLEDGEMENTS

Our appreciation goes to Dr. Ishak Bello (MD) and the staff of MILCOPAL dairy company, Kaduna, Mallam Mahmud of Bacterial Zoonoses laboratory and all the Fulani herds' men for their co-operation and assistance during the study.

REFERENCES

- AKLILU, E., ZUNITA, Z., HASSAN, L. AND CHENG, C H. (2013). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among veterinary students and personnel at a veterinary hospital in Malaysia. *Veterinary Microbiology*, **164** (3–4): 352–358.0
- BALA, H. K., IGWE, J. C., OLAYINKA, B. O., OLONITOLA, O. S. AND ONAOLAPO, J. A. (2016). Molecular characterization of methicillin resistant *Staph. aureus* from poultry farms in Kano State, Nigeria. *Journal of Bioscience and Biotechnology Discovery*, **1**: 74-82
- BRESSLER, A.M., WILLIAMS, T., CULLER, E. E., ZHU, W., LONSWAY, D., PATEL, J. B. AND NOLTE, F. S. (2005). Correlation of Penicillin Binding Protein 2a Detection with Oxacillin Resistance in *Staphylococcus aureus* and Discovery of a Novel Penicillin Binding Protein 2a Mutation. *Journal of Clinical Microbiology*, **43** (9): 4541–4544.
- CABRAL, K.G., LAMMLER, C., ZSCHOCK, M., LANGONI, H., DE SA, M. E. P., VICTORIA, C. AND DA SILVA, A. V. (2004). Pheno and genotyping of *Staphylococcus aureus* isolated from bovine milk samples from Sao Paola State, Brazilian. *Canadian Journal of Microbiology*, **50**: 901-909.
- CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI) (2014). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement*. Vol. 34 No. 1, CLSI document M100-S24

- (ISBN 1-56238-897-5 [Print]; ISBN 1-56238-898-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2014.
- DECLECG, P., PETRE, D., GOIRDTS, B AND VOSS, A. (2008). Complicated community-acquired soft tissues infection by MRSA from porcine origin. *Infection*, 36: 590-592
- DENIS, O., SUETENS, C., HALIM, M., CATRY, R., RAMBOUR, I., DISPAS, M., WILLEM, G., GORDTS, B., BUTAYE, P. AND STRUELENS, M. J. (2009). MRSA ST398 in swine farm personnel, Belgium. *Emerging Infectious Diseases*, 15: 1098-1101.
- DINWELL, R. T. LESLIE, K. E., SCHUKKEN, Y. H., SARGENT, Y. H. AND TIMMS, L. L. (2003). Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactations dairy cows. *Canadian Veterinary Journal*, 44 (5) 413-416.
- ERDEM AND TÜKYILMAZ, S. (2013). Molecular typing of methicillin-resistant *Staphylococcus aureus* strains isolated from cows and farm workers. *Kafkas University Veterinary of Derg*, 19: 963-968.
- FLETCHER, B. AND DAN, C. (1996). "Africa". *Sir Banister Fletcher's a History of Architecture*. Architectural Press, Pp. 1466.
- ARAN, K. P., GOLDDEN, S. M., BORUD, D., JAWHIR, S., BENDER, J. B. AND SREEVATSAN, S. (2012). Prevalence and characterization of *Staphylococcus aureus* including methicillin-resistant *Staphylococcus aureus* isolated from bulk tank milk from Minnesota dairy farms. *Journal of Clinical Microbiology*, 50 (3): 688-695.
- HELAL, G. T. Y., EL-ENBAAWY, M. I. AND MODERN, S. A. N. (2015). Genetic Expression of *Mec A* Gene in Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains of Animal and Human Samples. *Journal of Microbiology Research*, 5 (3): 77-83
- HUBER, H., KULLER, S., GIEZENDENNER, N., STEPHAN, R. AND ZWEIFEL, C. (2010). Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, livestock and in foods of animal origin. Switzerland and Eurosurveillance, 15: 1-4
- ITRAKAMHAENG, M., KOMUTARIN, T., PIMPUKDEE, K AND AENGWAMI, W. (2012). Incidence of enterotoxin-producing MRSA in bovine mastitis cases, bulk tank milk. *Journal of Animal and Veterinary Advances*, 11 (5): 655-661.
- JAPONI, A., ALBORZI, A., ORAFA, F., RASOULI, M. AND FARSHAD, S. (2004). Distribution patterns of methicillin resistance genes (*mec A*) in *Staphylococcus aureus* isolated from clinical specimens. *Iranian Biomedical Journal*, 8: 173-178.
- JEVONS, M. P. (1961). Celbenin-resistant staphylococci. *British Medical Journal*, 1: 124-125.
- JOSHI, L. R., TIWARI, A., DEVKOTA, S. P., KHATIWADA, S., PAUDYAL, S AND PANDE, K. R. (2014). Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Dairy Farms of Pokhara, Nepal. *International Journal of Veterinary Science*, 3 (2): 87-90.
- JUHANSZ-KASZANYITZKY, E., TÁNOSI, S., SOMOGYI, R., DÁN, Á., VAN DER ĞRAAF-VAN BLOOS, L., VAN DJUIJKEREN, E. AND WAGENAAN, A. (2007). MRSA transmission between cows and humans. *Emerging Infectious Diseases*, 13: 630-632.
- KADUNA STATE (2010). Alias: Centre of Education. Retrieved from: <http://www.kadunastate.gov.ng>.
- KHANNA, T., FRIENDSHIP, R., DEWEY, C. AND WEESE, J. S. (2008). Methicillin-resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary Microbiology*, 128: 298303.
- KASIULEVIČIUS, V., ŠAPOKA, V. AND FILIPA VIČIŪTĖ, R. (2006). Sample size calculation in epidemiological studies.

- Gerontologija*, 7 (4): 225–231.
- KREUSUKON, K., FESCH, A., KRAUSHAAR, B., ALT, K., MÜLLER, K., KRÖMKER, V., ZESSIN, K. H., KÄSBOIHRER, A. AND TENHAGEN, B. A. (2012). Prevalence, antimicrobial resistance of *Staphylococcus aureus* strains from bulk tank milk. *Journal of Dairy Science*, **95** (8): 4382-4388.
- LEE, J. H. (2003). Methicillin (Oxacillin)-resistance *S. aureus* strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology*, **69**: 6489-6494.
- LEWIS H. C., MØLBAK, K., REESE, C., AARESTRUP, F. M., SELCHAU, M., SØRUM, M. AND SKOV, R. L. (2008). Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. *Emerging Infectious Diseases*, 14: 1383-1389.
- LIVERMORE, D. M. (2000). Antibiotic resistance in staphylococci. *International Journal of Antimicrobial Agents*, 16 Suppl 1: S3-10.
- MAGIORAKOS, A. P., SRINIVASAN, A., CAREY, R. B., CARMELI, Y., FALAGAS, M. E., GISKE, C. G., HARBARTH, S., HINDLER, J. F., KAHLMETER, G., OLSSON-LILJEQUIST, B., PATERSON, D. L., RICE, L. B., STELLING, J., STRUELENS, M. J., VATOPOULOS, V., WEBER, J. T. AND MONNET, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbial Infection*, **18**: 268–281.
- MEKIBIB, B., FURGASA, M., ABUNNA, F., MEGERSA, B. AND REGASSA, A. (2010). Bovine mastitis: Prevalence, risk factors and major pathogens in dairy farms of Holeta Town, Central Ethiopia. *Veterinary World*, **3** (9): 397-403
- MIRZAEI, H., TOFIGHI, A., SARABI, H. K. AND FARAJI, M. (2011). Prevalence of methicillin resistant *Staphylococcus aureus* in raw milk and dairy products in Sarab by culture and PCR techniques. *Journal of Animal and Veterinary Advances*, **10** (23): 3107-3111.
- NORMANNO, G. A. (2005). Coagulase positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *International Journal of Food Microbiology*, **9**: 73-79
- OMOSHABA, E. O., OJO., O. E., SOFELA, O. AND ONIFADE, O. I. (2018). Prevalence and antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* in raw milk and soft cheese (*wara*) sold in Abeokuta, Nigeria. *Sokoto Journal of Veterinary Sciences*, **16** (1): 1-8.
- PATERSON, G. K., MORGAN, F. J. E., HARRISON, E. M., PEACOCK, S. J., PARKHIL, J., ZADOKS, R. N. AND HOLMES, A. (2013). Prevalence and properties of *mec C* methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. *Journal of Antimicrobial Chemotherapy*, 10: 1-5.
- PEREIRA, V., LOPES, C., CASTRO, A., SILVA, J., GIBBS, P. AND TEIXEIRA, P. (2009). Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiology*, 26: 278-282.
- QUINN, P. J., CARTER, M. E., MARKEY, B. K. AND CARTEY, G. R. (1994). Clinical Veterinary Microbiology, Section 2: Bacteriology, 8: *Staphylococcus* species, 118-126, Mosby-Year Book Europe Limited, London
- RAHIMI, F., BOUZARI, M., VANDYOUSEFI, J., MALEKI, Z., KASHANI, S. S. AND DAVOUDI, S. (2008). Analysis of antibiotic resistance and detection of *mec A* gene in *Staphylococcus aureus* isolated from hospital and medical laboratories in Tehran. *Iranian Biomedical Journal*, 21: 64-74
- REINOSO, E. B., EL-SAYED, A., LÄMMLER, C., BOGRINI, C. AND ZSCHÖCH, M. (2008). Genotyping *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. *Microbiology Research*, 163: 314-322.

- RESOURCE INVENTORY MANAGEMENT SYSTEMS (1990). Population of animals in Nigeria, RIMS Report, London, P. 43.
- STASTKOVA, Z., KARPISKOVA, S. AND KARPISKOVA, R. (2009). Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at goat breeding farm. *Veterinarni Medicina*, **54** (9): 419-426.
- SULEIMAN, A. B., UMOH, V. J., KWAGA, J. K. P. AND SHAIBU, S. J. (2012). Prevalence and antibiotic resistance profiles of Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from bovine mastitic milk in Plateau State, Nigeria. *International Research Journal of Microbiology*, 2 (8): 264-270.
- TAMAGNINI, L. M., SOUSA, G. B. GONZALEZ, R. D. AND BUDE, C. A. (2006). Microbial characteristics of crottin goat cheese made in different seasons. *Small Ruminant Research*, 66: 1745-180.
- TÜRKYILMAZ, S., TEKBIYIKI, S., ORYASIN, E. AND BOZDOGAN, B. (2010). Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. *Zoonoses and Public Health*, **57**: 197-203
- UMARU, G. A., KABIR, J., UMOH, V. J., BELLO, M. AND KWAGA, J. K. P. (2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *International Journal of Drug Research and Technology*, 3 (3): 67-75
- UNNERSTAD, H. E., BENGTSSON, B., AF RANTZIEN, M. H. AND BÖRJESSON, S. (2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) containing *mec C* in Swedish dairy cows. *Acta Veterinaria Scandinavica*, 55: 6-15.
- VAN DUIJKEREN, E., BOX, A. T. A., HERCH, M. E. O. C., WANNET, W. . B. AND FLUIT, A. C. (2004). Methicillin-resistant staphylococci isolated from animals. *Veterinary Microbiology*, **103** (1-2): 91-97
- VIRGIN, J. E., VAN SLYKE, T. M., LOMBARD, J. E. AND ZADOKS, R. N. (2009). Methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk, *Dairy Science*, **92**: 4988-4991.
- WEESE, J. S. (2005). Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals *Journal of American Animal Hospital Association*, **41**: 150-157.