

Full Length Research Paper

Occurrence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in fresh milk and milk products in Nasarawa State, North-Central Nigeria

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Methicillin resistant *Staphylococcus aureus* (MRSA) keeps emerging as a pathogen of significant public health concern. In this study, the occurrence and antibiogram of MRSA from fresh milk and milk products sold in Nasarawa State, north-central Nigeria, were determined. A total of 180 samples comprising of fresh milk, bulk milk, *nono* and *kindirmo* were collected and examined for the presence of antibiotic-resistant phenotypes of MRSA using standard microbiological procedures and molecular confirmation using species-specific 23S rDNA Polymerase chain reaction (PCR). Also, the presence of *mecA* gene was determined from confirmed MRSA strains using PCR. The cumulative occurrence of MRSA as obtained from this study was 5.00% (9/180); with 7.14, 6.06, 4.55 and 2.94% occurrence in bulk milk, *nono*, *kindirmo* and fresh milk respectively. With respect to the sampling locations, 2.86, 6.89, 7.14, 3.57, 6.45 and 3.45% occurrence of MRSA were recorded from Nasarawa, Keffi, Akwanga, Wamba, Lafia and Doma local government areas respectively. There was no significant difference ($P>0.05$) in the occurrence of MRSA in both the sample types and sampling locations. All the 9 MRSA strains examined showed 100% resistance to ampicillin and amoxicillin/clavulanic acid. This study recorded 5 antibiotic resistance patterns among the MRSA strains with multiple antibiotic resistance (MAR) index of 0.3 and above. Only 3 of the 9 MRSA strains isolated harboured *mecA* gene. This study provides baseline data on the public health risks associated with the consumption of milk products sold in Nasarawa State, Nigeria.

Key words: Methicillin-resistance, Milk, Nigeria, public health, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus has been implicated in many diseases of animals and humans, and the pathogenicity of this bacterium is mainly due to a combination of

genetic factors mediating virulence, invasive ability, ability to evade the immune system of the host, and antibiotic resistance (Chua et al., 2014). Milk and milk products,

especially those produced from raw milk under poor hygienic conditions serve as potential vehicles for the transmission of foodborne pathogens including antibiotic-resistant strains of *S. aureus* (Kadariyaet al., 2014).

Methicillin-resistant *S. aureus* (MRSA), including the livestock-associated methicillin-resistant *S. aureus* (LA-MRSA), has been isolated from dairy products such as raw milk and cheese globally (Peton and Le Loir, 2014). *Nono* and *kindirmo* are locally fermented milk products which are commonly produced and sold by Fulani pastoralists in Nigeria, especially in the northern part of the country. Since these products are produced under doubtful hygienic conditions, they could serve as vehicles for the transmission of MRSA.

In recent years, the spread of antibiotic-resistant bacterial pathogens, particularly multi-drug resistant zoonotic pathogens have raised increasing global concern (Ikeagwu et al., 2008). Related to this concern is the fact that, the use of antimicrobial agents in both human and veterinary medicine is promoting the steady rise in the prevalence of these antibiotic-resistant pathogens globally (Umaru et al., 2013). These antibiotic-resistant strains can be shed into the environment and consequently contaminate milk and milk products (Lee, 2003). MRSA harbours *mecA* gene (located on the staphylococcal cassette chromosome *mec* (SCC*mec*)) which codes for the production of an altered penicillin-binding protein (PBP2a or PBP2') instead of the normal PBP that has a low affinity for beta-lactam antibiotics (Kwon et al., 2006; Scott and Van Duijkeren, 2010). The polymerase chain reaction (PCR) has high sensitivity and specificity in detecting methicillin resistance in *S. aureus* and is not affected by culture conditions such as temperature, pH, and the sodium chloride (NaCl) content of the growth medium which make the phenotypic determination of methicillin resistance in *S. aureus* vague (Zamani et al., 2007). Data on the occurrence and antibiotic susceptibility of MRSA in Nasarawa State, north-central Nigeria is scarce. Consequently, this study sought to determine the occurrence, antibiotic resistance patterns, and detection of *mecA* by PCR technique in MRSA isolated from fresh milk and milk products in Nasarawa State, north-central Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted in Nasarawa State, North-Central Nigeria. Nasarawa State has with 13 Local Government Areas (LGA) and is situated between latitude 7° 40' 0"N and 9° 40' 0"N, and longitude 7° 0' 0"E and 9° 30' 0"E. It is bounded by Taraba State to the southeast, Kaduna State to the north, Plateau State to the northeast, Kogi State to the southwest, and to the south and

west by Benue State and the Federal Capital Territory (FCT), Abuja.

Sample size determination and sample collection

In this study, previously reported *S. aureus* prevalence of 12.60% (Umaru et al., 2013) was used for the determination of the sample size in the equation described by Naing et al. (2006). A total of 180 samples of fresh cow milk (34) and milk products consisting of bulk milk (14), locally fermented milk, *nono*(66) and locally-pasteurized milk, *kindirmo*(66) were randomly collected from each of the selected LGA including Akwanga and Wamba (Nasarawa North), Keffi and Nasarawa (Nasarawa West), and finally Lafia and Doma (Nasarawa South) respectively between May to October, 2018. Each of the collected sample was separately placed in sterile plastic bags, stored in cool box with ice and immediately transported to the microbiology laboratory of the Ahmadu Bello University, Zaria, Nigeria for microbiological analyses.

Isolation and Identification of *S. aureus*

A loopful of pre-enriched broth of each sample (25ml) diluted in 225ml of 1% buffered peptone water, homogenized and incubated for 18 h, and then inoculated onto the surface of prepared Baird-Parker agar (BPA) plates (Oxoid, Basingstoke, England) supplemented with 5% egg yolk tellurite emulsion (Baird-Parker, 1962). After 24 h incubation at 37°C, discrete colonies were selected based on their cultural and morphological features, and then sub-cultured onto selective media and nutrient agar for identification via biochemical tests (Patrick et al., 2013). *S. aureus* ATCC 25923 strain was used as a positive control.

Biochemical tests

The conventional biochemical tests carried out to identify the suspected *S. aureus* colonies were Catalase test, haemolysis on blood agar, slide coagulase test, and DNase test (Japoni et al., 2004). Also, the Microgen® Staphylococci Identification (STAPH identification) kit was used for the identification of *S. aureus* following the manufacturer's instruction.

Molecular characterization of the *S. aureus* strains

The genomic DNA was extracted by boiling method as previously described by Akanbi et al. (2017). The concentration and the purity of extracted DNA from each strain was determined (Perez-Roth et al., 2001) and then used as DNA templates for the PCR. PCR reaction was performed using species-specific amplification of 23S rDNA (1250bp). The reaction was performed according to the method described by Shuiepet al. (2009). The forward and reverse primers used and PCR conditions were: 'F-ACGGAGTTACAAAAGGACGAC' and 'R - AGCTCAGCCTTAACGAGTAC' (Shuiepet et al., 2009) (Inqaba, South Africa). The reaction mixture (25 µl) consisted of 1.0 µl of each primer (forward and reverse) (10 pmol/µl), 0.8 µl dNTP (10 mM), 3.0 µl of 10x Taq buffer (Promega®, USA), with a final concentration of 1.8 µl MgCl₂, (Promega®, USA), 0.1 µl Taq polymerase (5U/µl) (Promega®, USA), 2.5 µl of genomic DNA, and final volume was adjusted to 25 µl by adding the remaining

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Table 1. The occurrence of MRSA in relation to the type of milk sample collected from Nasarawa State, north-central Nigeria.

Type of milk sample	No. examined	No. positive (%)	χ^2	p-value
<i>Nono</i>	66	4(6.06)	0.646	0.886
Bulk Milk	14	1(7.14)		
Fresh Milk	34	1(2.94)		
<i>Kindirmo</i>	66	3(4.55)		
Total	180	9(5.0)		

Volume of nuclease free-water. Amplification was carried out with thermocycler (Gene AMP[®] PCR System 9700, AB Applied Biosystems, Singapore) with an initial denaturation at 94°C for 5 min, followed by 37 cycles of denaturation at 94°C for 40s, annealing temperature of primers was 64°C for 60 s, extension at 72°C for 75 s and the final extension was done at 72°C for 7 min. A total of 10 µl of the amplified PCR products were solved by electrophoresis in 1.5% agarose gel (Amresco Bioscience, USA) with Trisacetate electrophoresis buffer (TAE, 4.0 mmol/L Tris 1 mmol/L EDTA, pH 8.0) at 100 V for 30 min, stained with ethidium bromide and finally visualized and documented under UV transilluminator (BIO-RAD Molecular Imager[®] Gel Doc[™], Canada). The image was stored in a computer for further analysis.

Antibiogram of *S. aureus* strains

The Kirby-Bauer agar disc diffusion method as described by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2013) was used for the determination of the antibiogram of the *S. aureus* strains. *S. aureus* strains were examined against eleven antibiotics with the following concentrations: amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), gentamicin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), imipenem (10 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), sulphamethoxazole/trimethoprim (25 µg), vancomycin (30 µg), and tetracycline (30 µg) (Oxoid, England). Based on the diameter zone of growth inhibition, the results were expressed as sensitive, intermediate, or resistant using CLSI breakpoint (CLSI, 2013). *S. aureus* ATCC 25932 was used as the quality control standard strain.

Determination of multiple antibiotics resistance (MAR) Index of the *S. aureus* strains

The MAR index was determined for each of the strain examined using the formula:

$$\text{MARI} = x/y,$$

Where 'x' is the number of antibiotics to which the strain display resistance, and 'y' is the total number of antibiotics to which the test strain had been evaluated for sensitivity (Tula et al., 2013).

Molecular detection of *mecA* gene in the MRSA phenotypes

The genomic DNA was extracted and the concentration and purity determined as earlier described. PCR reaction was performed to detect the presence of *mecA* in strains that phenotypically exhibited resistance to cefoxitin (methicillin) according to the method described by Strommenger et al. (2003). The presence of *mecA* gene was detected in the methicillin-resistant isolates using the forward and reverse primers: 'F-AAAATCGATGGTAAAGGTTGGC'

and' R-AGTTCTGCAGTACCGGATTTGC' (Inqaba, South Africa) that are expected to yield a PCR product of approximately 533 bp (Strommenger et al., 2003). The PCR was carried out in a 50 µl of reaction mixture with a PCR buffer containing 200 µM concentration of each deoxynucleosidetriphosphate (dNTP), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 1.25 unit of Taq polymerase (Promega, Germany), 0.25 µM concentration of each primer and 2.5 µl of DNA template. Amplification was carried out in a thermocycler (Gene AMP[®] PCR System 9700, AB Applied Biosystems, Singapore). DNA amplification was carried out for 50 cycles in 50 µl of reaction mixture as follows: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products (10 µl) were analysed in 1.5% agarose gel. The gel containing amplified DNA was stained with ethidium bromide solution (10 mg/ml) for 10 min and destained with distilled water for 10 min. The 533 bp PCR products were visualized and documented under UV transilluminator (BIO-RAD Molecular Imager[®] Gel Doc[™], Canada). DNA from a known *mecA*-carrying *S. aureus* ATCC33591 strain was used as PCR positive control.

Statistical analyses

The data obtained from this study were analyzed using the chi-square test which was used to determine the statistically significant difference between the occurrences of MRSA in the different sample types and sampling areas. Comparisons were considered statistically significant when P-values < 0.05.

RESULTS

The cumulative occurrence of MRSA as obtained from this study was 5.00% (9). From the samples examined, 4 (6.06%), 1 (7.14%), 3 (4.55%) and 1 (2.94%) from *nono*, bulk milk, *kindirmo* and fresh milk were respectively contaminated with MRSA (Table 1) with respect to sampling location, Nasarawa, Keffi, Akwanga, Wamba, Lafia, and Doma local government areas were found to have 2.86, 6.89, 7.14, 3.57, 6.45 and 3.45% occurrence of MRSA from all the samples examined (Table 2). The results of antibiotic susceptibility pattern of the 9 MRSA strains examined using 11 antibiotics are presented in Table 3. The results revealed that 6 (66.7%) of the isolates were susceptible to sulphamethoxazole/trimethoprim, 8 (88.9%) were susceptible to vancomycin, 7 (77.8%) were susceptible to chloramphenicol, 4 (44.4%) were susceptible to erythromycin, 9 (100%) were

Table 2. The prevalence of MRSA in fresh milk and milk products in relation to the sampling Locations.

LGAs	No. examined	No. positive (%)	χ^2 - value	p-value
Nasarawa	35	1(2.86)	1.233	0.942
Keffi	29	2(6.89)		
Akwanga	28	2(7.14)		
Wamba	28	1(3.57)		
Lafia	31	2(6.45)		
Doma	29	1(3.45)		
Total	180	9(5.0)		

LGAs – Local Government Areas.

Table 3. The antibiotic susceptibility profile of MRSA isolated from fresh milk and milk products in Nasarawa State, north-central Nigeria.

Antibiotics	Disc conc. (μ g)	N= 9		
		S (%)	I (%)	R (%)
Ampicillin	10	0(0.0)	0(0.0)	9(100.0)
Amoxicillin/ clavulanic acid	30	0(0.0)	0(0.0)	9(100.0)
Cefoxitin	30	0(0.0)	0(0.0)	9(100.0)
Gentamicin	30	9(100.0)	0(0.0)	0(0.0)
Chloramphenicol	30	7(77.8)	2(22.2)	0(0.0)
Vancomycin	30	8(88.9)	1(11.1)	0(0.0)
Ciprofloxacin	5	9(100.0)	0(0.0)	0(0.0)
Erythromycin	15	4(44.4)	3(33.3)	2(22.2)
Imipenem	10	8(88.9)	1(11.1)	0(0.0)
Tetracycline	30	3(33.3)	2(22.2)	4(44.4)
Sulphamethoxazole/ Trimethoprim	25	6(66.7)	1(11.1)	2(22.2)

Table 4. The Antibiotic Resistance Patterns of MRSA Isolated from Fresh and Fermented Milk in Parts of Nasarawa State, Nigeria.

No. of antibiotics	Resistance Pattern	No. (%) of isolates	LGA
3	Amp, Amo, Fox	4(44.4)	NS, KF, AK, AK
4	Amp, Amo, Fox, Tet	2(22.2)	LF, WM
5	Amp, Amo, Fox, Tet, Sul,	1(11.1)	KF
5	Amp, Amo, Fox, Ery, Tet	1(11.1)	LF
5	Amp, Amo, Fox, Ery, Sul	1(11.1)	DM

AMP = Ampicillin, Amo = Amoxicillin/clavulanic acid, Fox = cefoxitin, Tet = Tetracycline, Ery= Erythromycin, Sul = Sulphamethoxazole/trimethoprim, NS = Nasarawa, KF = Keffi, AK = Akwanga, WM = Wamba, LF = Lafia, DM = Doma.

susceptible to ciprofloxacin and gentamicin, 8 (88.9%) were susceptible to imipenem and 3 (33.3%) were susceptible to tetracycline respectively (Table 3). However, whereas all the MRSA isolates were resistant to ampicillin and amoxicillin/clavulanic acid, 4 (44.4%) were resistant to tetracycline while 2 (22.2%) were resistant to both sulphamethoxazole/trimethoprim and erythromycin. The results obtained further showed that imipenem, gentamicin, ciprofloxacin, chloramphenicol,

vancomycin, and sulphamethoxazole/trimethoprim were the antibiotics to which the isolates examined were most susceptible.

The results of the antibiotic resistance patterns of the 9 MRSA isolates obtained from fresh milk and milk products in Nasarawa State, north-central Nigeria is as presented in Table 4. A total of 5 antibiotics resistance phenotypes were obtained with varying multiple resistance types including 3, 4 and 5 different antibiotics

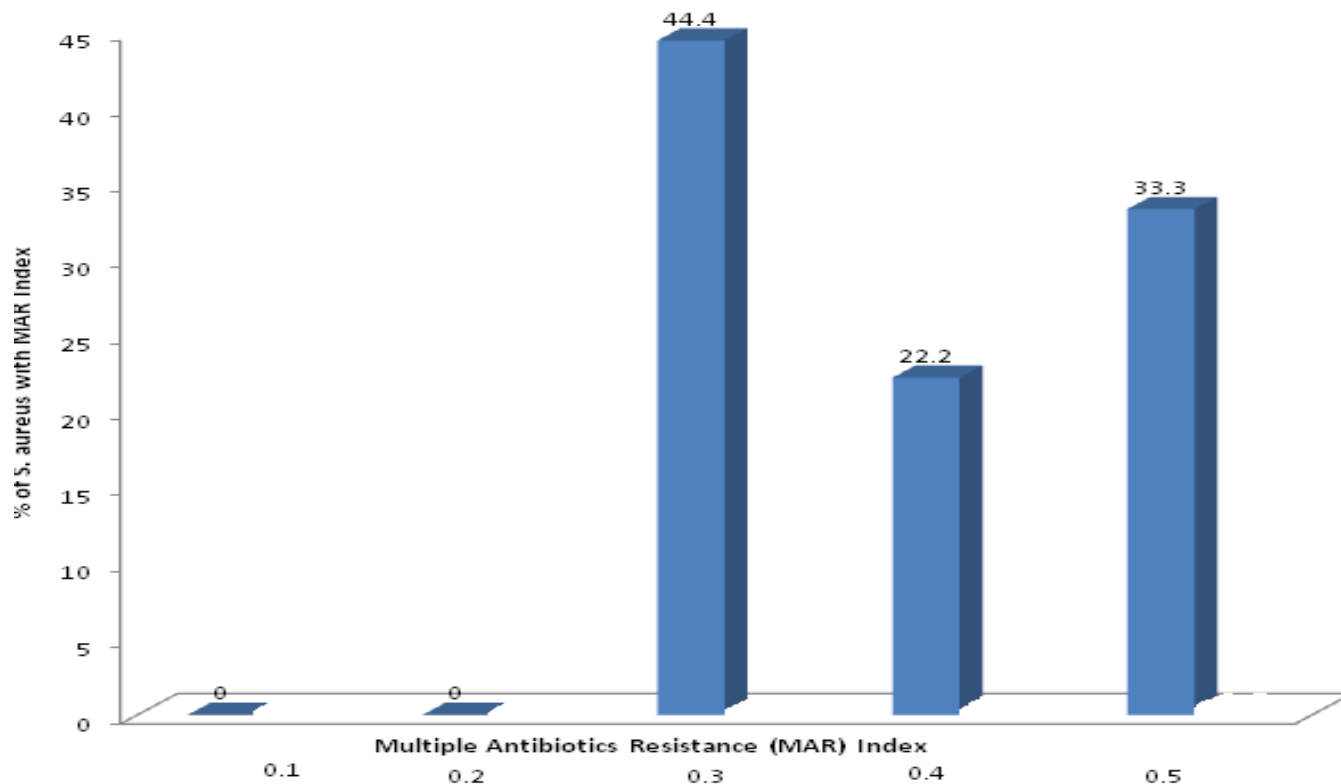


Figure 1. Multiple Antibiotic Resistance (MAR) Index of MRSA isolated from fresh milk and milk products Nasarawa State, north-central Nigeria.

combinations. All the isolates examined were resistant to 3 or more antibiotics. The highest frequency of multi-drug resistance patterns (as seen in three different isolates) were found to be made of 5 different antibiotics. Furthermore, the MAR indexes of the MRSA isolates obtained from this study are 0.3 and above (Figure 1). The results of the amplification of the species-specific 23S rDNA of the presumptive *S. aureus* isolates was molecularly confirmed with bands showing the amplicon size of 1250 bp positive for *S. aureus* (Figure 2). Furthermore, the presence of *mecA* gene was detected in only 3 of the 9 MRSA isolates examined by PCR. The 3 MRSA isolates with *mecA*-positive as evidenced by the amplification of the *mecA* gene specific amplicon size of 533bp were: AKN1, NSN5, KFK12 (Figure 2).

DISCUSSION

Over the years, milk and milk products have been known as vehicles for the transmission of bacterial pathogens to man (Revathi et al., 2012). The contamination of food with antibiotic-resistant pathogens poses a significant global public health threat. The determinants of antibiotic resistance in these pathogens can be transferred to other bacteria of clinical significance. The cumulative occurrence of MRSA in fresh milk and milk products in

Nasarawa State, north-central Nigeria as recorded in this study was 5.0%. However, higher MRSA occurrence of 12.14 and 12.60% from fresh milk and milk products in Kaduna and Zaria (which are major cities in north-western Nigeria) were previously reported (Umaru et al., 2013; Usman and Mustapha, 2016) respectively. Similarly, our result was also lower than the 8.70% prevalence of MRSA recorded from fresh and fermented milk in parts of Kaduna State, Nigeria (Okpo et al., 2016). Furthermore, previous findings from other countries showed higher MRSA occurrence of 56, 55.26, 32 and 25.53% from dairy products in Turkey, Kenya, Algeria, and Bangladesh respectively (Patrick et al., 2013; Gundogan and Avci, 2014; Jahan et al., 2015; Chaalal et al., 2016). The paucity of information on MRSA in milk and milk products in the study area made it difficult to make any comparison and to assess the level of *S. aureus* in dairy products in the areas studied. This study is the first comprehensive research conducted to carefully examine the occurrence of MRSA in dairy products in different municipal areas of Nasarawa State, north-central Nigeria, where these products are readily marketed and consumed.

The isolation of MRSA from milk products is a cause for public health concern because these products are regularly consumed in the study area without considering the associated health hazards. The findings of this

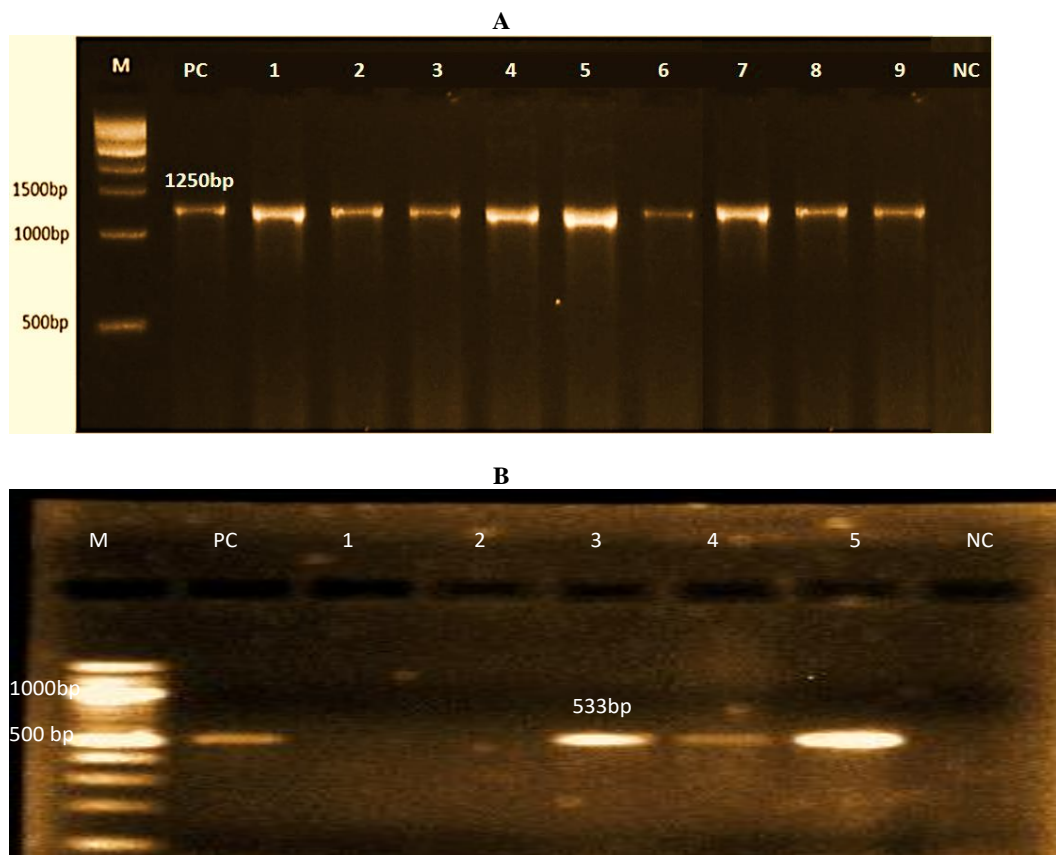


Figure 2. (A) Agarose gel electrophoresis of PCR product after amplification of 23S rDNA gene at of *S. aureus* isolates at 1250 bp. Lane M: 1,500-bp DNA ladder; PC, Positive control; Lanes 2-9: positive isolates at 1250 bp; NC, Negative control. (B) Agarose gel electrophoresis of PCR products after amplification of *mecA* gene at 533 bp. Lane M: 100-1000 bp DNA ladder. Lane PC: Positive control; Lanes 3, 4, and 5 positive isolates at 533bp; Lane NC: Negative control

study also support the assertion that dairy products are one of the major vehicles for the transmission of MRSA to man. There was non statistically significant difference ($P>0.05$) recorded in the occurrence of MRSA among the fresh milk and milk products examined in this study. This can be attributed to the similar and uniform procedures employed during milking, processing, production and handling of these milk products within the study area.

Out of the nine MRSA strains isolated from the current study, one each was detected from fresh and bulk milk samples examined while four and three were isolated from *nono* and *kindirmo* samples respectively. This trend of MRSA occurrence is in contrast with the previous report of Umoh (1989) stating that fermented foods are generally not good media for the survival and growth of *S. aureus*. The occurrence of this organism in these locally processed foods may imply recontamination during and/or after processing. Notwithstanding, proper heat treatment and refrigeration can minimize the chances of contamination with *S. aureus*. It has also been observed that during the heat treatment of milk to

produce *kindirmo*, the temperature does not rise enough to achieve effective pasteurization. The occurrence of *S. aureus* in fresh and bulk milk as recorded in this study may be attributed to the presence of subclinical mastitis in the milked cows poor sanitary practices during milking or use of unclean milking utensils. The main source of *S. aureus* in milk is the udder of infected cows which could be transferred via the milkers' hands, milking utensils, towels, and the environment (Radostitsi et al., 1994). *S. aureus* can adapt and survive in the udder of cow and establish chronic and sub-clinical infections. From the udder, it can be shed into the milk which serves as a primary source of infection to individuals who consume unpasteurized milk.

The antibiotic susceptibility profile revealed that strains of MRSA examined were highly susceptible to ciprofloxacin (100%), gentamicin (100%), vancomycin (88.9%), imipenem (88.9%), and chloramphenicol (77.8%). The high susceptibility of these isolates to ciprofloxacin, gentamicin, and chloramphenicol was in consonance with other findings (Okpo et al., 2016;

Rodrigues et al., 2017), who recorded high susceptibility of MRSA isolated from dairy products to these three antibiotics in areas in Kaduna State, north-western Nigeria and in Brazil respectively. The high performance of these antibiotics could be attributed to their small molecular sizes, a factor that enhances their solubility in diluents, thus enhancing their penetration power through the cell wall into the cytoplasm of the target organism where they exert their effects (Okpo et al., 2016). This also agrees with the assertion of Maillard (2002) who opined that the high efficacy of these antibiotics in MRSA strains may be attributed to their molecular sizes.

Similarly, this study recorded a high level of MRSA strains susceptibility (88.9%) to vancomycin, a glycopeptide. This finding is not surprising because vancomycin is rarely used in the treatment of diseases in livestock in the study area. Also, the administration of vancomycin as chemoprophylaxis and antibiotic growth promoter which could further lead to resistance among bacteria as a result of selective pressure is not a known practice within Nasarawa State, Nigeria. In consonance with the findings of the present study, Suleiman et al. (2012) and Rodrigues et al. (2017) opined that the non-use of vancomycin for routine chemoprophylaxis and therapy in an area could result in high vancomycin susceptibility of strains of *S. aureus* isolated from such locality. The findings from this study further agrees with the report of Alianet et al. (2012) in Iran who recorded 0.0% resistance to vancomycin among *S. aureus* isolated from dairy products. However, our finding differs starkly with the report of Usman and Mustapha (2016), and Umaru et al. (2013) who reported 66.7% and 42.6% vancomycin resistance in MRSA strains isolated from fresh milk and milk products in Nigeria. The disparity between our findings and the aforementioned could be as a result of contamination of the milk with vancomycin-resistant *S. aureus* derived from human sources.

All the 9 MRSA strains examined from this study were resistant to amoxicillin/clavulanic acid and ampicillin. This could be attributed to the use and misuse of these antibiotics in the study area. This finding is not surprising because outside the hospital environment, people have easy access to various antibiotics at any drug store without any prescription from qualified personnel. The findings of Anueyiagu and Isiyaku (2015) who reported 100% resistance of *S. aureus* isolated from dairy products in Jos, Plateau State, Nigeria and also in Bangladesh (Jahan et al., 2015) agrees with the results obtained from this study. Moreover, β -lactam antibiotics including ampicillin and amoxicillin/clavulanic acid are commonly used as dry-cow treatment. This may also have contributed to the increasing resistance among *S. aureus* strains to these drugs due to selective pressure. *S. aureus* resistant to one β -lactam drug can further develop resistance to other β -lactams drugs because they have the same mechanism of activity.

The relatively high level to tetracycline resistance as

observed in this study could be attributed to tetracycline being the most commonly available antibiotic that is used as a growth promoter and routine prophylaxis in livestock management in Nigeria (Olatoye, 2010). Tetracycline resistance is a cause for immense concern considering that it is a first-line drug in Nigeria. Also, individuals with cases of gastrointestinal infections in Nigeria and most developing countries readily purchase tetracycline over-the-counter for self-medication (Chigor et al., 2010). Furthermore, MRSA strains from dairy products have been reported to show high instances of 55.5 and 40% resistance to tetracycline in Nigeria (Usman and Mustapha, 2016) and Ethiopia (Tessema, 2016). This trend is a cause for concern in both human medicine and livestock production due to the existing emergence of bacterial strains resistant to major antibiotics. The use of antibiotics either as prophylaxis or growth promoters in food animals are now regarded as the major avenue for the spread of resistant bacteria through the food chain to humans result in severe human infections (Phillips et al., 2004).

The relatively high resistance exhibited by MRSA strains to erythromycin in the study area reflects its frequent use and abuse. Higher levels of 85.7 and 76% resistance among *S. aureus* isolated from dairy products have been reported in Nigeria (Anueyiagu and Isiyaku, 2015) and Iran (Mirzaei et al., 2012) respectively. Also, the relatively high levels of resistance to sulphamethoxazole/trimethoprim as recorded in this study is baffling, considering the fact that the drug is not routinely used in veterinary practice in Nigeria. This may suggest cross-contamination of the dairy products by handlers with the drug-resistant pathogens. Furthermore, mixed fermentation is known to occur in local milk products including *nono* and *kindirmo*. As a result of such uncontrolled fermentation process, different organisms can be involved at different times, hence, transferring determinants of antibiotic resistance between them.

Food is an important medium through which the transfer of determinants of antibiotic resistance among bacteria occur. Such transfer can occur by means of residues of antibiotics in foods, transmission of resistant pathogens, or by the ingestion of resistant foodborne pathogens (Pereira et al., 2009).

Multi-drug resistance is defined as the resistance of an isolate to two or more antibiotics (Olayinka et al., 2004). All the nine MRSA strains isolated from this study showed multidrug resistance to the antibiotics tested at different percentages. The isolates were resistant to a combination of three, four, and five antibiotics tested. Isolates obtained from Keffi, Akwanga, and Lafia showed higher frequencies of multi-drug resistance. The multi-drug resistance recorded among all the MRSA strains examined in this study agrees with other findings (Umaru et al., 2013; Anueyiagu and Isiyaku, 2015; Chaalal et al., 2016; Tessema, 2016), who reported cases of multi-drug resistance among MRSA strains isolated from dairy

products in Zaria and Jos (Nigeria), Ethiopia, and Algeria respectively. Multi-drug resistance in *S. aureus* may be attributed in part, to the spread of mobile genetic elements like plasmids, transposons, and integrons that may confer resistance to numerous antimicrobial agents (Zhao et al., 2001). According to Aarestrup (1995), the determinants of multi-drug resistance are capable of being disseminated in a region or between regions as a result of antibiotic selective pressure in either livestock or humans. Empirical evidence abounds which indicate that drug-resistant strains of bacteria can be transmitted to humans via food (Khachatourians, 1998; Revathi et al., 2012; Tessema, 2016).

This study recorded 5 antibiotic resistance patterns among the MRSA strains examined which varies from the 25 antibiotic resistance patterns recorded among *S. aureus* strains isolated from dairy products in Bahir Dar, Ethiopia (Shiferaw and Ahmad, 2016). The disparity in the antibiotic resistance patterns of MRSA strains recorded in the present study and the previous results recorded in Ethiopia could be as a result of the different levels of abuse of antibiotics in the two areas. All the MRSA strains examined in this study had a MAR index of 0.3. MAR index gives an indirect indication of the probable source of an organism. An organism originates from an environment with antibiotics abuse when its MAR index is greater than 0.2 (Furtula et al., 2013).

Results from this study revealed ciprofloxacin, gentamicin, vancomycin, imipenem, and chloramphenicol as drugs of choice for MRSA infections in the study area since majority of the isolates were susceptible to them. The public health significance of the present findings is that antibiotic-resistant strains of *S. aureus* from milk products (or dairy animals) may be transmitted from the food chain to humans through occupational exposure, contact or wastewater run-off from production sites. More so, in Nigeria, the indiscriminate use of antibiotics in livestock production could have also contributed immensely to the patterns of antibiotic resistance recorded in this study.

The detection of *mecA* gene is the gold standard for the determination of MRSA from either clinical, food or environmental samples. Three (AKN1, NSN5, and AKF2) out of the nine MRSA strains examined harboured *mecA* gene with an approximate amplified PCR product size of 533 bp, indicating that the phenotypic resistance exhibited by the strains to ceftiofur (methicillin) was due to the possession of *mecA* gene. This finding concord with the report of Umaru et al. (2013), who detected *mecA* gene in only four out of the 18 MRSA strains isolated from raw milk and milk products in Nigeria. Our findings also agree with the results obtained by Suleiman et al. (2012) who also reported the presence of *mecA* gene in only 2 out of the 26 MRSA strains isolated from milk in Plateau State, Nigeria. Similarly, out of the four MRSA strains isolated from milk in Brazil, Rodrigues et al.

(2017) reported the presence of *mecA* gene in only two of the four MRSA strains examined.

Conversely, however, the finding of this study differs with the results obtained by Usman and Mustapha (2016) in which no *mecA* gene was detected in all of the nine MRSA strains isolated from skimmed milk and yogurt in Kaduna metropolis, Nigeria. In the same vein, *mecA* gene was not detected in all of the 15 MRSA strains isolated from raw milk in Malaysia (Shamila-Syuhada et al., 2016).

The disparity between the results obtained in this study and those of the other researchers mentioned above can be attributed to the assertion of Lee et al. (2004), who posited that phenotypic resistance of *S. aureus* to ceftiofur (methicillin) could vary depending on cellular growth conditions. Phenotypic expression of resistance to methicillin in MRSA varies, and each strain has a characteristic profile of the proportion of bacterial cells that grow at specific concentrations of methicillin (Plata et al., 2013). It has also been observed that PCR detection of *mecA* gene does not always give indisputable results (Adesida et al., 2005). Some MRSA strains have been found to be *mecA*-negative in PCR, but resistant to ceftiofur (methicillin). Furthermore, some MRSA strains have also been reported to be *mecA*-positive but susceptible to ceftiofur (methicillin) (Olonitola et al., 2007). The absence of *mecA* in MRSA strains could also be an indication of the potential presence of 'modified *S. aureus*' (MODSA). MODSA possess modified penicillin-binding proteins (PBPs) which is a different classical mechanism of resistance to methicillin in MRSA (Bhutia et al., 2012). In this study, a negative correlation was found between the phenotypic resistance to ceftiofur (methicillin) and PCR results for the detection of *mecA* gene that code for resistance to methicillin. The antibiotic susceptibility profile of the MRSA strains isolated from fresh milk and milk products showed high levels of resistance to ampicillin, amoxicillin/clavulanic acid and tetracycline. This finding is of great public health concern because these antibiotics are commonly used in Nigeria either therapeutically in human and veterinary medicine or as growth promoters and prophylaxis in animal production. This study finally established the presence of *mecA* gene in only three out of the nine MRSA strains isolated from fresh milk and milk products in the study area. There was also an indication of a negative correlation between the phenotypic resistance to ceftiofur (methicillin) and the presence of *mecA* gene which have been established to code for resistance to methicillin in MRSA strains.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest.

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