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Antibiotic resistant phenotypes of *Staphylococcus aureus* isolated from fresh and fermented milk in parts of Nasarawa State, Nigeria

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This work was aimed at determining the occurrence and antibiogram of *Staphylococcus aureus* isolated from fresh and fermented milk samples in parts of Nasarawa State, Nigeria. A total of 180 samples comprising of fresh raw milk, bulk milk, *nono*, and *kindirmo* were collected over a period of 6 months (May to October, 2017). Standard microbiological procedures were employed in the isolation, identification, characterisation, and determination of the antibiogram of *S. aureus* from the milk samples. Characterisation of the *S. aureus* isolates was by morphological, biochemical characteristics using conventional methods, Microgen® STAPH-ID kits. Confirmed isolates were tested for susceptibility or resistance to a panel of 11 commonly used antibiotics using the agar disc diffusion technique. Out of the 180 milk samples examined, 9 *S. aureus* were isolated giving a prevalence of 5.0%. The occurrence of *S. aureus* was higher in *nono* (12.1%) and *kindirmo* (10.6%) than in fresh raw milk (5.9%). The high occurrence of *S. aureus* in *nono* disproved the assertion that fermented foods are not good media for the survival and growth of *S. aureus*. The antibiotic susceptibility profile of the *S. aureus* isolates indicated all of the nine isolates were completely resistant to cefoxitin, ampicillin, and amoxicillin/clavulanic acid. The isolates were moderately resistant to erythromycin (22.2%), sulphamethoxazole/trimethoprim (22.2%), and tetracycline (44.4%). Five antibiotic resistance patterns were recorded among the isolates. All of the isolates had a multiple antibiotics resistance (MAR) index of 0.3 and above, an indication of possible antibiotic misuse in the areas studied.

Key words: Milk, *Staphylococcus aureus*, antibiotic resistant phenotypes, Nasarawa State, Nigeria.

INTRODUCTION

Raw milk and fermented milk of various types are produced and consumed as supplement to normal meals

in homes and even for sale (Maduka et al., 2013). Traditionally produced dairy products especially those

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produced from raw milk under neglected hygienic conditions, are potential vehicles for the transmission of different foodborne pathogens especially toxigenic *Staphylococcus aureus* (Kadariya et al., 2014). *S. aureus*, including those associated with animals have been frequently recovered from raw milk and milk products worldwide (Peton and Le Loir, 2014). Despite the fundamental roles that milk and milk products play in human nutrition, they serve as vehicles for the transmission of many bacterial pathogens to man. For example, in Europe, milk and other dairy products are found to be responsible for 5% of staphylococcal outbreaks (Bianchi et al., 2014). Health risk to consumers can be associated with milk due to the presence of zoonotic pathogens and antimicrobial drugs residues (Vyletělová et al., 2011). Milk quality can be lowered by a number of factors such as contamination during and after milking, and the presence of udder infections. Pathogenic microorganisms in milk can be derived from the cow itself, the human hand, or the environment (Esron et al., 2005).

Antibiotic usage has become common place in human medicine and animal production. The extensive use of antibiotics in both human medicine and agriculture particularly in disease prevention and growth enhancement in animal production is a considerable cause of the selection and prevalence of antibiotic resistant microorganisms (Jamali et al., 2015). The use of antimicrobial agents is associated with the risk of inducing resistance to antimicrobial agents among bacterial pathogens and transmission of resistance bacteria to humans via the food chain (Jamali et al., 2015).

Food contamination with antibiotic resistant pathogens poses a major public health threat as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance (Jamali et al., 2014). Contamination of food products with microorganisms influence considerably, the safety of the products, endanger the health of consumers, lower their shelf life resulting in foodborne infections, intoxications, and economic losses due to food spoilage (Shiferaw and Ahmad, 2016). Milk is considered as a good substrate on which *S. aureus* grow and produce enterotoxins (Korpysa-Dzirba and Osek, 2011). *S. aureus* has been reported as one of the most common causative agents of food poisoning associated with the consumption of raw milk and milk products (Spanu et al., 2012). Contamination of food stuff occurs directly from infected food-producing animals or may result from poor hygiene during production processes or the storage and retail of food since humans also harbours microorganisms (Vázquez-Sánchez et al., 2012).

The hygienic standard of milk may be assessed based on the level of contamination with *S. aureus* and studying the antibiotic resistant phenotypes of this bacterial pathogen will provide valuable information on antibiotic

usage and infection control strategy in animal production. Considering the aforementioned points, this study was conducted with the aim of isolating, characterizing and determining the antibiotic resistant patterns of *S. aureus* from fresh and fermented milk samples in parts of Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out in Nasarawa State, Nigeria. Two Local Government Areas were selected in the three Senatorial Zones in the State viz: Akwanga and Wamba (Nasarawa North); Lafia and Doma (Nasarawa South); Keffi and Nasarawa (Nasarawa West) (Plate 2). Nasarawa State is situated between latitude 70° 40 and 90° 40, and longitude 70° 0 and 90° 30. The State has thirteen Local Government Areas and is bounded to the north by Kaduna State, Plateau State to the northeast, Taraba State to the southeast, Benue State to the south, Kogi State to the southwest, and the Federal Capital Territory (FCT), Abuja, to the west (Plate 1). These positions were taken using Taiwan-made Etrex® high sensitive Geographic Positioning System (GPS) receiver.

Study design

Sample size

The sample size was determined using the 12.6% prevalence of *S. aureus* as reported by Umaru et al. (2013). The sample size was determined by using the equation described by Naing et al. (2006):

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where n is the sample size;

P is the prevalence from a previous study = 12.6% = 0.126;

Z is the standard normal distribution at 95% confidence interval = 1.96;

d is the absolute desired precision at 5% = 0.05.

Therefore,

$$n = \frac{(1.96)^2 \times 0.126 \times (1-0.126)}{(0.05)^2} = \frac{0.4231}{0.0025} = 169 \text{ samples}$$

Sample collection

For the purpose of this study, the sample size was rounded up to 180. Thirty cow milk and milk products samples were randomly collected from each of the Local Government Areas selected for this study viz: Akwanga and Wamba (Nasarawa North); Lafia and Doma (Nasarawa South); Keffi and Nasarawa (Nasarawa West) respectively from May to October, 2017.

Proportionate sampling method was used in collecting fresh raw milk samples from lactating cows at the accessible Fulani settlements. This was done by taking 50% of the number of lactating cows in a herd at the settlements. On the whole, 34 fresh raw milk samples were collected. Herds were visited during milking time, where 30 ml of composite fresh cow milk samples were

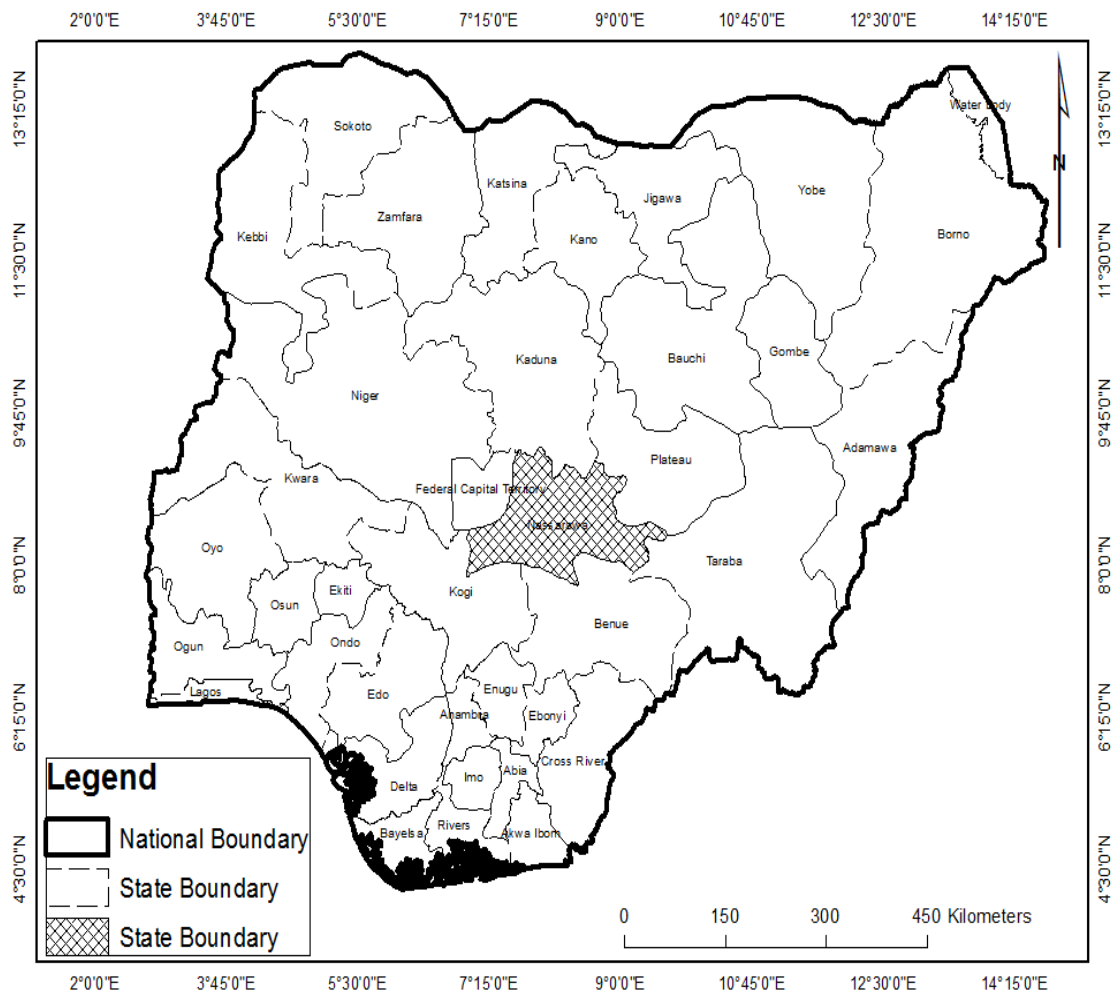


Plate 1. A Map of Nigeria showing the Position of Nasarawa State.

collected directly from the milking cows and placed into sterile bottles. Each sample (30 ml) was collected into sterile screw-capped plastic bottles and labelled appropriately.

Fourteen bulk milk samples were collected from the accessible Fulani settlements in each town selected for this study. The bulk fresh milk samples were collected after the milk have been collected and pooled. Twenty two *nono* and locally-pasteurised milk (*kindirmo*) samples were randomly purchased from vendors in the towns selected for this study. All samples were placed in separate sterile plastic bags to prevent spillage and cross contamination. Samples were stored in a cooler with ice blocks and then transported to the Biochemical Laboratory of the Institute for Agricultural Research (IAR), Samaru, Zaria and the Postgraduate Students' Laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, for proximate and microbiological analyses respectively.

Isolation and identification of *S. aureus*

All samples were inoculated with the aid of a sterile wire loop onto the surface of prepared Baird-Parker agar plates (Oxoid, Basingstoke, England) supplemented with 5% egg yolk tellurite

emulsion (Baird-Parker, 1962). Representative colonies were selected after incubation at 37°C for 24 h based on the appearance of presence of black colonies on the medium which occur as a result of the ability coagulase-positive staphylococci to reduce tellurite, and clear zones of lypolysis which is due to the lecithinase of staphylococci that break down the egg yolk. Discrete colonies were further sub-cultured on to freshly prepared plates of the selective media and nutrient agar plates for biochemical tests and identification (Patrick et al., 2013). *S. aureus* ATCC 25923 strain was used as a positive control.

Observation of colony morphology and characteristics

Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media that was used for the isolation and Gram reaction.

Biochemical tests

The conventional biochemical tests carried out to identify the suspected *S. aureus* colonies were Catalase test, slide coagulase

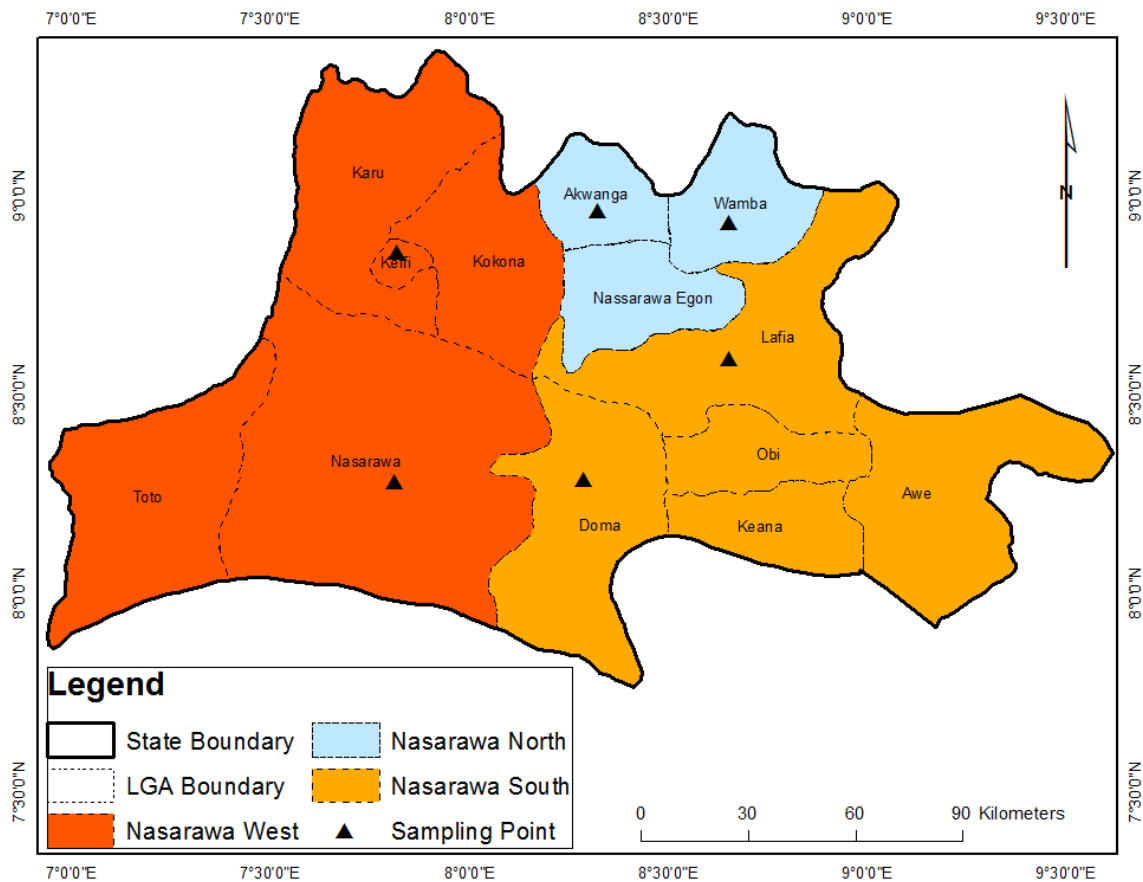


Plate 2. Map of Nasarawa State showing the sampling areas.

test, haemolysis on blood agar, and DNase test (Japoni et al., 2004).

Microgen® staphylococci identification (STAPH identification) kits

The Microgen Staph-ID system comprises of a single microwell test strip containing 12 standardised biochemical substrates which have been selected on the basis of extensive computer analysis that is, each well contains dehydrated substrates, namely: nitrate, sucrose, tetrahalose, mannitol, n-acetyl glucosamine, mannose, turanose, N-acetyl glucosamine, β -glucosidase, β -glucuronidase, urease, arginine, and 1-pyrrolidonyl- α -naphthylamide (www.microgenproducts.com UK). A colour change occurs if the individual substrates are metabolised by the organism during incubation, or after addition of specific reagents.

Determination of the antibiogram of the *S. aureus* isolates

The antibiogram of the isolates was determined using the Kirby-Bauer agar disc diffusion method as described by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2016). The isolates were tested against a panel of eleven antibiotics with the following concentrations: ampicillin (10 μ g), amoxicillin/clavulanic acid (30 μ g), chloramphenicol (30 μ g), imipenem (10 μ g), erythromycin (15 μ g), gentamicin (30 μ g), ciprofloxacin (5 μ g), ceftioxin (30 μ g),

vancomycin (30 μ g), sulphamethoxazole/trimethoprim (25 μ g), and tetracycline (30 μ g) (Oxoid, England). The determination of sensitive, intermediate, or resistant isolates depend on the diameter zone of growth inhibition of Clinical Laboratory Standards Institute's (CLSI) breakpoint (CLSI, 2016). *S. aureus* ATCC 25932 obtained from the National Veterinary Research Institute (NVRI), VOM, Plateau State, Nigeria was included in each batch analysis as the quality control standard strain.

Determination of minimum inhibitory concentration (MIC) of vancomycin

The MIC of vancomycin against the isolates was determined by broth microdilution method as recommended by the Clinical Standards Institute (CLSI, 2016). Standard powders of the antibiotic (vancomycin) were obtained from GlaxoSmithKline Pharmaceutical Companies. The MIC of vancomycin against the isolates was determined using the procedure described by Wayne (2002). Readings were taken and recorded according to the guidelines of CLSI (2016).

Determination of multiple antibiotics resistance (MAR) index of the *S. aureus* isolates

The multiple antibiotics resistance (MAR) index was determined for each of the isolate using the formula: $MARI = x/y$, where 'x' is the

Table 1. The occurrence of *S. aureus* in relation to the type of milk samples collected from parts of Nasarawa State, 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)		

Table 2. The prevalence of *S. aureus* in fresh and fermented milk in relation to the local government areas sampled.

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)		

Key: LGAs – Local Government Areas.

number of antibiotics to which the isolate display resistance, and 'y' is the total number of antibiotics to which the test organism had been evaluated for sensitivity (Olayinka et al., 2004; Tula et al., 2013).

Statistical analyses

The chi-square test was used to determine the statistically significant difference between the occurrence of *S. aureus* and the different sample types, and also the occurrence of *S. aureus* in fresh and fermented milk in the different sampling areas. $P \leq 0.05$ was considered statistically significant for all comparisons.

RESULTS AND DISCUSSION

Table 1 shows the occurrence of *S. aureus* in relation to the type of milk samples collected from parts of Nasarawa State, Nigeria. Out of the 66 *nono* samples collected and analysed in the course of this study, four were found to be contaminated with *S. aureus* giving a prevalence of 6.06%; of the 14 bulk milk samples analysed, one was found to be positive for *S. aureus* giving a prevalence of 7.14%; of the 66 *kindirmo* samples examined, three were found to be positive for *S. aureus* giving a prevalence of 4.55%; and of the 34 fresh milk samples examined, one was found to be contaminated with *S. aureus* giving a prevalence of 2.94% (Table 1). The results obtained from this study revealed that, *S. aureus* were present in fresh and fermented milk in parts of Nasarawa State, Nigeria. This is of public health

significance since it is a commonly recovered pathogen in outbreaks of food poisoning attributed to dairy products (Junaidu et al., 2011). The occurrence of *S. aureus* (5.0%) in the study area is an indication of defective or absence of public health measures and poor sanitary habits among the people that are concerned with milking, milk handling, and transportation as these have been documented to be factors that predisposes milk to contamination with pathogens (Akram et al., 2013). The use of traditional method of *nono* and *kindirmo* production also exposes the products to bacteria found on the hands and clothes of the people that are concerned with the production and also in the containers used. The unsanitary conditions of the places where the products (*nono* and *kindirmo*) are marketed might have also contributed to their contamination.

Table 2 shows the prevalence (%) of *S. aureus* obtained from fresh and fermented milk in the six sampling areas selected for this study. Out of the 180 samples collected in the course of the study, nine (5.0%) were found to be contaminated with *S. aureus*. Out of the 35 samples collected from Nasarawa Local Government Area, one yielded positive result for *S. aureus* with a prevalence of 2.86%; of the 29 samples collected from Keffi Local Government Area, two yielded positive results for *S. aureus* giving a prevalence of 6.89%; of the 28 samples each collected from Akwanga, two yielded positive results for *S. aureus* with a prevalence of 7.14%; of the 28 samples each collected from Wamba, one yielded a positive result for *S. aureus* with a prevalence

of 3.57%; of the 31 samples collected from Lafia Local Government Area, two were found to be contaminated with *S. aureus* with a prevalence of 6.45%; and out of the 29 samples collected from Doma Local Government Area, one was found to be contaminated with *S. aureus* giving a prevalence of 3.45% (Table 2). The percentage occurrence of *S. aureus* in fresh and fermented milk in parts of Nasarawa State, recorded in this study was 5.0%, which was lower than the 12.6 and 12.14% recorded by Umaru et al. (2013) and Usman and Mustapha (2016), in studies conducted to determine the occurrence of *S. aureus* in fresh and fermented milk in Kaduna and Zaria, respectively. It was also lower than the 8.7% prevalence recorded by Okpo et al. (2016) in fresh and fermented milk in parts of Kaduna State, Nigeria. Higher occurrence of *S. aureus* of 32, 56, 25.53 and 55.26% were reported by Patrick et al. (2013), Gundogan and Avci (2014), Jahan et al. (2015), and Chaalal et al. (2016). Higher occurrence of the organism in dairy products in Kenya, Turkey, Bangladesh, and Algeria respectively. The paucity of information on *S. aureus* in milk and foods in general 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.

The isolation of *S. aureus* from fresh and fermented milk is a cause for public health concern because many people in the area consume the products. The findings of this work lend credence to the assertion that, dairy products is one of the major vehicle for the transmission of *S. aureus* to man. No statistically significant difference ($p > 0.05$) was found using chi-square in the occurrence of *S. aureus* in fresh and fermented milk with respect to the different sample types collected in the course of this study, indicating that, the milk samples might have been exposed to the same levels of contamination. This may be due to similar handling procedures employed during milking, milk collection, and processing and production of fermented milk (*nono* and *kindirmo*). This trend of occurrence of *S. aureus* is in contrast with was documented by Umoh (1989) that fermented foods are not good media for the survival and growth of *S. aureus*. The occurrence of the organism in these processed foods implies recontamination during and/or after processing. Proper heat treatment and refrigeration can minimise the chances of contamination with *S. aureus*. It has been observed that, during the heat treatment of milk to make *kindirmo*, the temperature does not rise up enough to achieve effective pasteurisation. The occurrence of *S. aureus* in fresh and bulk milk in this study may be attributed to the presence of sub-clinical mastitis in the milked cows, poor sanitary practices during milking, and 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 (Radostitis et al., 1994). *S. aureus* can adapt to and survive in the udder of cow and

establish chronic and sub-clinical infections. From the udder, it is shed into the milk which serves as a primary source of infection to individuals who drink unpasteurised milk.

The results antibiotics susceptibility profile of the nine *S. aureus* isolated from fresh and fermented milk in parts of Nasarawa State using 11 antibiotics are as presented in Table 3. The results shows that, six (66.7%) of the isolates were susceptible to sulphamethoxazole/trimethoprim, 8(88.9%) of the isolates were susceptible to vancomycin, seven (77.8%) were susceptible to chloramphenicol, four (44.4%) were susceptible to erythromycin, nine (100%) were susceptible to gentamycin and ciprofloxacin, eight (88.9%) were susceptible to imipenem, while only three (33.3%) were susceptible to tetracycline (Table 3). However, nine (100%) of the *S. aureus* isolates were resistant to cefoxitin, ampicillin, and amoxicillin/clavulanic acid, four (44.4%) were resistant to tetracycline, two (22.2%) were resistant to both erythromycin and sulphamethoxazole/trimethoprim. The results shows that, gentamycin, ciprofloxacin, imipenem, vancomycin, chloramphenicol, and sulphamethoxazole/trimethoprim were the antibiotics to which the isolates were most susceptible; whereas, erythromycin, and tetracycline were the antibiotics to which the isolates were less susceptible. All the nine *S. aureus* isolates obtained in this study were resistant to cefoxitin, an indication that they are all methicillin-resistant *S. aureus* (MRSA) strains. Cefoxitin is used as a surrogate test for oxacillin to check whether *S. aureus* isolates are MRSA or not (CLSI, 2016). The antibiotic susceptibility profile of the *S. aureus* isolates showed that, they were highly susceptible to gentamycin (100%), ciprofloxacin (100%), imipenem (88.9%), vancomycin (88.9%), and chloramphenicol (77.8%).

The high susceptibility of the isolates to gentamycin, ciprofloxacin, and chloramphenicol was in consonance with the findings of Okpo et al. (2016) and Rodrigues et al. (2017) who recorded high susceptibility of *S. aureus* to isolated from dairy products to the three antibiotics in parts of Kaduna State, Nigeria in Brazil, respectively. The high performance of these antibiotics to could be attributed to their small molecular sizes - a factor that enhances the 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 agrees with the assertion of Mailard (2002) who opined that, the high efficacy of antibiotics may be attributed to their molecular sizes.

High level of susceptibility (88.9%) of the *S. aureus* obtained in this study to vancomycin was observed. None of the isolates was found to be resistant to the antibiotic (vancomycin). This finding is not surprising because vancomycin is rarely used in the treatment of diseases in livestock and in routine chemoprophylaxis in the study area which could lead to resistance among bacteria as a result of selective pressure. This result is in consonance

Table 3. The antibiotic susceptibility profile of *S. aureus* Isolated from fresh and fermented milk in parts of Nasarawa State.

Antibiotics	Disc Conc. (µg)	N= 9		
		S (%)	I (%)	R (%)
Ampicillin	10	0(0.0)	0(0.0)	9(100.0)
Amoxicillin/ clavulanic cid	30	0(0.0)	0(0.0)	9(100.0)
Cefoxitin	30	0(0.0)	0(0.0)	9(100.0)
Gentamycin	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)

with the findings of Suleiman et al. (2012) and Rodrigues et al. (2017) who opined that, the non-use of vancomycin for routine chemoprophylaxis and therapy in an area can result in *S. aureus* exhibiting high susceptibility to it. This finding also agrees with the results of Alian et al. (2012) in Iran who recorded 0% resistance among *S. aureus* isolated from dairy products. However, this finding contrasted starkly with that of Umaru et al. (2013) and Usman and Mustapha (2016), who reported 42.6 and 66.7% resistance of *S. aureus* isolated from fresh and fermented milk in to vancomycin in Kaduna and Zaria, respectively. The disparity between the findings of the present study and the aforementioned could be as a result of contamination of the milk with vancomycin-resistant *S. aureus* derived from human sources. The present study observed that all the nine *S. aureus* isolates were completely resistant to ampicillin and amoxicillin/clavulanic acid. 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. This agrees with the findings of Anueyiagu and Isiyaku (2015) who reported 100% resistance of *S. aureus* isolated from dairy products in Jos, Plateau State, Nigeria, and Jahan et al. (2015) in Bangladesh. β -lactam antibiotics are commonly used as dry-cow treatment. This might have contributed to the increasing resistance among *S. aureus* strains to the drugs due to selective pressure. *S. aureus* resistant to one β -lactam drug can develop resistance to β -lactams because they have the same mechanism of activity.

Relatively high levels of resistance of *S. aureus* to tetracycline (44.4%), erythromycin (22.2%), and sulphamethoxazole/trimethoprim (22.2%) were recorded in this study. The relatively high level to tetracycline 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). This finding is a cause for concern considering the fact that, tetracycline is a first-line drug in Nigeria. This is one drug that people with cases of gastro-intestinal infections in most developing countries readily purchase over-the-counter for self-medication (Chigor et al., 2010). This was in consonance with the findings of Usman and Mustapha (2016), and Tessema (2016), who reported 55.5 and 40% resistance of *S. aureus* isolated from dairy products in Kaduna State, Nigeria, and Ethiopia respectively. This trend is a cause for concern in human medicine and livestock disease management and production generally due to the existing emergence of bacterial strains that are resistant to major antibiotics. The use of antibiotics in food animals have been established to promote the spread of antibiotic-resistant bacteria via the food chain to humans resulting in human infections (Phillips et al., 2004). The relatively high level of resistance to erythromycin could be a reflection of the frequent use and misuse of the antibiotic in the study area. Higher levels of resistance of 76 and 85.7% among *S. aureus* isolated from dairy products have been reported Mirzaei et al. (2012) and Anueyiagu and Isiyaku (2015) in Iran and Kaduna State, Nigeria, respectively.

The relatively high level of resistance to sulphamethoxazole/trimethoprim in this study is baffling considering the fact that, the drug is not routine used in veterinary practice in Nigeria. This suggests cross contamination of the dairy products by handlers with the drug-resistant strains of the pathogen. Mixed fermentation is known to occur in dairy products like *nono* and *kindirmo* and as the fermentation process is uncontrolled and that different organisms can occur at different times, transfer of determinants of antibiotic resistance can occur between organisms. Food is an important medium through which the transfer of determinants of antibiotic resistance among bacteria occurs. Such transfer can occur by means of residues of antibiotics in foods, through the transfer of antibiotic-

Table 4. The antibiotic resistance patterns of *S. aureus* 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.

resistant foodborne pathogens, or through the ingestion of drug-resistant strains of the original food microflora, and transfer of transfer of antibiotic-resistance determinants in bacteria (Pereira et al., 2009).

The results of the antibiotic resistance patterns of the nine *S. aureus* isolates obtained from the fresh and fermented milk samples examined are as presented in Table 4 and the multiple antibiotics resistance (MAR) index of the nine isolates is as shown in Figure 1. Five antibiotic resistance phenotypes were obtained, all from the multiple resistance types with varying combinations of three, four and five antibiotics. No antibiotics resistance phenotype was found with a single or two antibiotics as all of the isolates were found to be resistant to three antibiotics and above. The highest frequency three (isolates showing resistance to a combination of antibiotics) was a found in a combination of five antibiotics. Multidrug resistance is defined as resistance of an isolate to two or more antibiotics (Olayinka et al., 2004). This finding is in consonance with the findings of Umaru et al. (2013), Anueyiagu and Isiyaku (2015), Tessema (2016), and Chaalal et al. (2016) who reported cases of multidrug resistance among *S. aureus* isolated from dairy products in Zaria, Jos, Ethiopia, and Algeria respectively. The isolates were resistant to a combination of three, four, five, and six of the antibiotics tested. Isolates obtained from Keffi, Akwanga, and Lafia, showed higher frequencies of multi-drug resistance. 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) and Levin et al. (1997), 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).

The five antibiotic resistance patterns among the *S. aureus* isolates recorded in this study varied with the nine and 25 antibiotic resistance patterns recorded among *S.*

aureus isolated from dairy products by Usman and Mustapha (2016), and Shiferaw and Ahmad (2016) in Kaduna State, Nigeria and Bahir Dar, Ethiopia, respectively. The disparity in the antibiotic resistance patterns of *S. aureus* isolates recorded in the present study and the one recorded in Ethiopia could be as a result of the different levels of use and misuse of antibiotics in the two areas. 100% of the *S. aureus* isolates obtained in this study had a MAR index of 0.3 and above. MAR index was calculated as the ratio of the number of antibiotics to which an organism is resistant, to the total number of antibiotics to which an organism is exposed (Furtula et al., 2013). MAR index gives an indirect suggestion of the probable source of an organism. MAR index greater than 0.2 is an indication that, an organism originates from an environment where there are no strict regulations regarding the use of antibiotics (Furtula et al., 2013). Residues of antibiotics have also been found in the tissues of food animals and their products (Kabir et al., 2004; Adesokan et al., 2013).

The public health significance of the findings of this study is that, antibiotic resistant strains of *S. aureus* from fresh and fermented milk (or dairy animals) may be transmitted to humans via the food chain, contact through occupational exposure, or wastewater run-off from *nono* and *kindirmo* production sites to the neighbourhood. Indiscriminate use of antibiotics in livestock production in the country could also have been responsible for the antibiotic resistance patterns recorded in this study.

Conclusion

The detection of *S. aureus* in fresh and fermented milk in parts of Nasarawa State, Nigeria, suggests that consumption of dairy products especially those that are produced using traditional methods constitute a hazard to consumers as the transmission of pathogens via foods has been well documented. The antibiotic susceptibility profile of the *S. aureus* isolates revealed high performance of gentamicin, ciprofloxacin, imipenem, vancomycin, and chloramphenicol, while relatively high

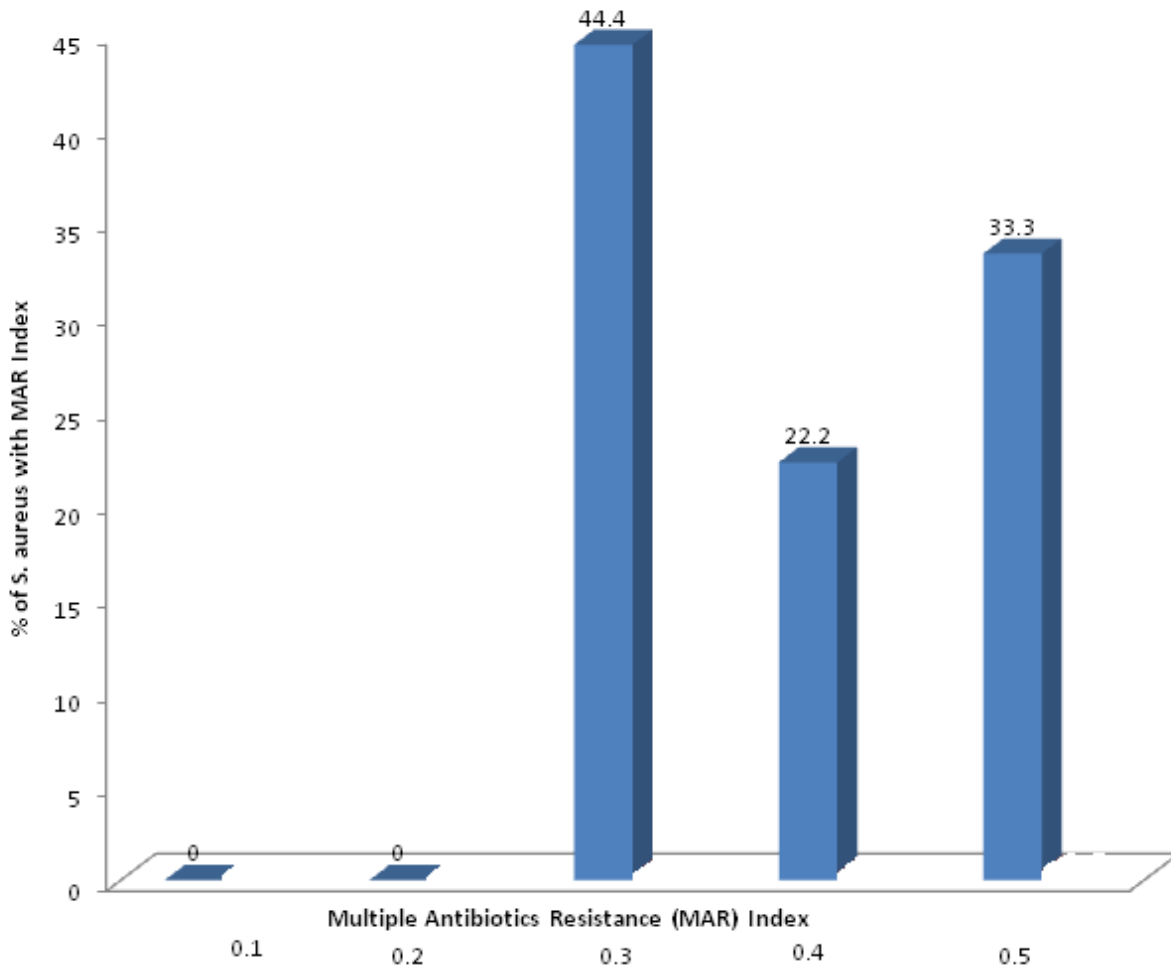


Figure 1. Multiple antibiotic resistance (MAR) Index of *S. aureus* isolated from fresh and fermented milk in parts of Nasarawa state, Nigeria.

levels of resistance to tetracycline was recorded. This is of public health concern because tetracycline is a commonly used antibiotic. The data obtained in this study suggests that, selection pressure imposed by the use of antibiotics whether therapeutically in human and veterinary medicine, or in routine chemoprophylaxis in livestock production is a key driving force in the promotion of antibiotic resistance in *S. aureus*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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