



## Prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in Nasarawa State, Nigeria

A Yakubu<sup>1\*</sup>, IO Abdullahi<sup>2</sup>, CZ Whong<sup>2</sup> & B Olayinka<sup>3</sup>

<sup>1</sup> Department of Science Laboratory Technology, Federal Polytechnic, Nasarawa, Nigeria

<sup>2</sup> Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

<sup>3</sup> Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

\*Correspondence: Tel.: +2348067834134; E-mail: aleeyaqu29@gmail.com

**Copyright:** © 2020

Yakubu *et al.* This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Abstract**

This study determined the prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* isolated from milk and milk products in 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. Standard microbiological procedures were employed in the isolation, identification, characterization and determination of the antibiogram of *S. aureus* from the milk samples. Characterization was achieved by morphological, biochemical characteristics using conventional methods and Microgen® STAPH-ID kits. The 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, nine (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 kind of water (well water) used for cleaning utensils at the Nono and Kindirmo selling points was found to be a risk factor associated with the occurrence of *S. aureus* in the products. All of the isolates were resistant to cefoxitin (100%), ampicillin (100%), and amoxicillin/clavulanic acid (100%). The isolates displayed various rates of resistance to erythromycin (22.2%), sulphamethoxazole/trimethoprim (22.2%), and tetracycline (44.4%). Five (5) antibiotic resistance patterns were recorded among the isolates an indication of different levels of use and misuse of antibiotics in the areas studied. The detection of *Staphylococcus aureus* in fresh and fermented milk in the areas studied suggests that consumption of dairy products especially those produced using traditional methods, constitute a hazard to consumers. It is recommended that since compliance with basic hygiene requirements is not guaranteed, hazard analysis and critical control points (HACCP) concepts should be seen as a part of an effective total hygiene concept at the selling points.

**Publication History:**

Received: 02-10-2019

Accepted: 24-01-2020

**Keywords:** Antibiotic susceptibility profile, Milk, Nasarawa State, Nigeria, Prevalence, *Staphylococcus aureus*

## Introduction

Raw milk and milk products of various types are consumed as supplement to normal meals in homes and even for commercial purposes (Maduka *et al.*, 2013). Traditionally produced milk products especially those 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). *Staphylococcus aureus*, including those associated with animals have been frequently recovered from raw milk and milk products worldwide (Peton & 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 (Vyletřlova *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 (Adams & Moss, 2011). Pathogenic microorganisms in milk can be derived from the cow itself, the human hand during handling, or the environment (Esron *et al.*, 2005).

Antibiotic use is commonplace 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 pressure and prevalence of antibiotic resistant bacteria (Jamali *et al.*, 2015). The use of antimicrobial agents is associated with the risk of inducing resistance to antimicrobial agents in 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 & Ahmad, 2016). Milk is considered as a good substrate on which *Staphylococcus aureus* grow and produce enterotoxins (Korpysa-Dzirba & Osek, 2011).

*Staphylococcus 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. Contamination could also occur during the storage and retail of foods 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 susceptibility profile and risk factors associated with the occurrence of this bacterial pathogen in foods like milk and milk products will provide valuable information on planning effective control and preventive measures of the pathogen. Considering the aforementioned points, this study was conducted with the aim of isolating, characterizing and determining the antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in parts of Nasarawa State, Nigeria.

## Materials and Methods

### Study area

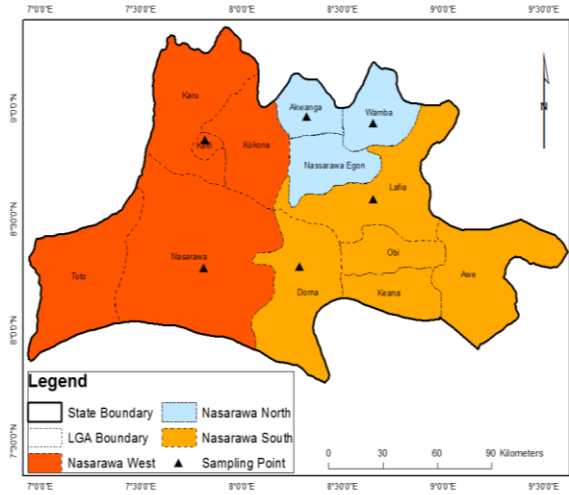
This study was carried out in Nasarawa State, Nigeria. Two (2) Local Government Areas were selected in each of the three Senatorial Zones in the State viz: Akwanga and Wamba (Nasarawa North); Lafia and Doma (Nasarawa South); Keffi and Nasarawa (Nasarawa West) (Figure 1). The State has thirteen (13) Local 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 (Figure 2). These positions were taken using Taiwan-made Etrex® high sensitive Geographic Positioning System (GPS) receiver. Nasarawa State is situated between latitude 70° 40 and 90° 40, and between longitude 70° 0 and 90° 30.

### 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):

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



**Figure 1:** Map of Nasarawa State showing the sampling areas



**Figure 2:** A map of Nigeria showing the position of Nasarawa State

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 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 composite fresh cow milk samples were collected directly from the milking cows and placed into sterile bottles. Each sample (30ml) 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 has been collected and pooled. Twenty-two *nono* and locally-pasteurized

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 cool box 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 *Staphylococcus 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). *Staphylococcus 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 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 standardized biochemical substrates which have been selected on the basis of extensive computer analysis i.e. each well contains dehydrated substrates, namely: nitrate, sucrose, tetrahalose, mannitol, N-acetyl glucosamine, mannose, turanose, N-acetyl glucosamine,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, urease, arginine, and 1-pyrrolidonyl- $\alpha$ -naphthylamide ([www.microgenproducts.com](http://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.

#### *Assessment of the risk factors associated with the occurrence of Staphylococcus aureus in Milk and Milk Products*

Structured questionnaires were used in investigating some risk factors that are associated with the occurrence of *Staphylococcus aureus* in milk and milk products.

#### *Determination of the antibiotic susceptibility profile 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 and Laboratory Standards Institute (CLSI) (CLSI, 2016). The isolates were tested against a panel of eleven antibiotics with the following concentrations: ampicillin (10 $\mu$ g), amoxicillin/clavulanic acid (30 $\mu$ g), chloramphenicol (30 $\mu$ g), imipenem (10 $\mu$ g), erythromycin (15 $\mu$ g), gentamicin (30 $\mu$ g), ciprofloxacin (5 $\mu$ g), ceftiofur (30 $\mu$ g), vancomycin (30 $\mu$ g), sulphamethoxazole/trimethoprim (25 $\mu$ g), and tetracycline (30 $\mu$ g) (Oxoid, England). The determination of sensitive, intermediate, or resistant isolates depended on the diameter zone of growth inhibition of CLSI breakpoint (CLSI, 2016).

*Staphylococcus 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 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).

#### *Statistical analysis*

The Chi-square test was used to determine the significant differences between the occurrence of *Staphylococcus aureus* in the different sample types analyzed, and the occurrence of *Staphylococcus aureus* in the different sampling areas, and the association between some risk factors and the occurrence of the bacterium in milk and milk products. P-values of 0.05 were considered significant for all comparisons.

## **Results**

Table 1 show the distribution of *Staphylococcus aureus* in fresh and fermented milk samples collected from the study areas. Although there were variations in the occurrence of the bacterium in the different sample types, no significant association ( $\chi^2 = 0.646$ ) was found between its occurrence and the different samples collected.

Table 2 show the occurrence of *Staphylococcus aureus* in fresh and fermented milk samples collected from the different Local Government Areas studied. No significant association ( $\chi^2 = 1.233$ ) was found between the occurrence of the bacterium and the different Local Government Areas sampled.

The results of the risk factors associated with the occurrence of *Staphylococcus aureus* in fresh and fermented milk are presented in Table 3. A significant association ( $\chi^2 = 62.219$ ) was found between of the bacterium in *nono* samples collected and the kind of water used for cleaning utensils at the product's selling points. Similarly, a significant association ( $\chi^2 = 63.119$ ) was found between the occurrence of *Staphylococcus aureus* in *kindirmo* and the kind of water used for cleaning utensils at the product's selling points. Higher occurrence of the bacterium

was found in *nono* and *kindirmo* samples collected from selling points with high street activities (mainly vehicular movement) compared to samples collected from selling points with low street activities.

The results antibiotic susceptibility profile of the nine (9) *Staphylococcus aureus* isolated from fresh and fermented milk in parts of Nasarawa State using 11 antibiotics are as presented in Table 4. The results show that, 66.7% of the isolates were susceptible to sulphamethoxazole/trimethoprim, 88.9% of the isolates were susceptible to vancomycin, seven 77.8% were susceptible to chloramphenicol, 44.4% were susceptible to erythromycin, 100% were susceptible to gentamicin and ciprofloxacin, 88.9% were susceptible to imipenem, while 33.3% were susceptible to tetracycline (Table 3). However, 100% of the *S. aureus* isolates were resistant to ceftiofur, ampicillin, and amoxicillin/clavulanic acid, 44.4% were resistant to tetracycline, 22.2% were resistant to both erythromycin and sulphamethoxazole/trimethoprim. All the nine (9) *S. aureus* isolates obtained in this study were resistant to ceftiofur, an indication that they are all methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The antibiotic susceptibility profile of the *S. aureus* isolates showed that, they were highly susceptible to gentamicin (100%), ciprofloxacin (100%), imipenem (88.9%), vancomycin (88.9%), and chloramphenicol (77.8%).

The results of the antibiotic resistance patterns of the nine (9) *Staphylococcus aureus* isolates obtained from the fresh and fermented milk samples examined are as presented in Table 5. Five (5) antibiotic resistance phenotypes were obtained, all from the multiple resistance types with varying combinations of three (3), (4), and five (5) antibiotics. No antibiotic resistance phenotype was found with a single or two antibiotics as all of the isolates were found to be resistant to three (3) antibiotics and above. The highest frequency (3) (isolates showing resistance to a combination of antibiotics) was a found in a combination of five (5) antibiotics.

**Discussion**

The results obtained from this study revealed that, *Staphylococcus 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 *Staphylococcus 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, transportation and sales as these have been documented to be factors that predisposes milk to contamination with pathogens (Akram *et al.*, 2013).

**Table 1:** Occurrence of *Staphylococcus aureus* in Relation to the Type of Milk Samples Collected from Parts of Nasarawa State, Nigeria

| Type of Milk Sample | No. Examined | No. Positive (%) | $\chi^2$ | 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.** Prevalence of *Staphylococcus aureus* in Fresh and Fermented Milk in relation to the Local Government Areas sampled

| LGAs     | No. examined | No. Positive (%) | $\chi^2$ - 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

**Table 3.** Risk Factors Associated with the Occurrence of *Staphylococcus aureus* in Fresh and Fermented Milk in Parts of Nasarawa State, Nigeria

| Risk factor  | No. Examined (%) | No. Positive (%) | $\chi^2$ | $p$ -value | Odds Ratio (95%CI) |
|--|------------------|------------------|----------|------------|--------------------|
| <b>Washing of teats and udder before milking</b>                                   |                  |                  |          |            |                    |
| Yes  | 6(17.65)         | 0(0.0)           | 0.221    | 0.638      | 0.964(0.898-1.036) |
| No   | 28(82.35)        | 1(2.94)          |          |            |                    |
| Total  | 34(100.0)        |                  |          |            |                    |
| <b>Milkers washing their hands before milking</b>                                  |                  |                  |          |            |                    |
| Yes  | 2(5.88)          | 0(0.0)           | 0.064    | 0.800      | 0.969(0.910-1.031) |
| No   | 32(94.12)        | 1(3.13)          |          |            |                    |
| Total  | 34(100.0)        | 1(2.94)          |          |            |                    |
| <b>Cleanliness of milk storage containers</b>                                      |                  |                  |          |            |                    |
| Satisfactory   | 4(21.43)         | 0(0.0)           | 0.431    | 0.512      | 0.900(0.732-1.107) |
| Unsatisfactory   | 10(71.43)        | 1(10.0)          |          |            |                    |
| Total  | 14(100.0)        | 1(7.14)          |          |            |                    |
| <b>Source of water for cleaning utensils at the <i>Nono</i> selling points</b>     |                  |                  |          |            |                    |
| Tap  | 0(0.0)           | 0(0.0)           | 62.219   | 0.000      | -                  |
| Vendors  | 48(72.73)        | 1(2.08)          |          |            |                    |
| Well   | 18(27.27)        | 3(16.67)         |          |            |                    |
| Total  | 66(100.0)        | 4(6.06)          |          |            |                    |
| <b>Source of water for cleaning utensils at the <i>Kindirmo</i> selling points</b> |                  |                  |          |            |                    |
| Tap  | 0(0.0)           | 0(0.0)           | 63.119   | 0.000      | -                  |
| Vendors  | 42(63.64)        | 1(2.38)          |          |            |                    |
| Well   | 24(36.36)        | 2(8.33)          |          |            |                    |
| Total  | 66(100.0)        | 3(4.55)          |          |            |                    |
| <b>Street activities at the <i>Nono</i> selling points</b>                         |                  |                  |          |            |                    |
| High   | 64(96.96)        | 4(6.25)          | 0.133    | 0.715      | 0.938(0.880-0.999) |
| Low  | 2(3.03)          | 0(0.0)           |          |            |                    |
| Total  | 66(100.0)        | 4(6.06)          |          |            |                    |
| <b>Street activities at the <i>Kindirmo</i> selling points</b>                     |                  |                  |          |            |                    |
| High   | 65(98.48)        | 3(4.62)          | 0.048    | 0.826      | 0.954(0.904-1.006) |
| Low  | 1(1.52)          | 0(0.0)           |          |            |                    |
| Total  | 66(100.0)        | 3(4.55)          |          |            |                    |

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 are marketed might have also contributed to their contamination.

The percentage occurrence of *Staphylococcus 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 & Mustapha (2016), in studies conducted to determine the occurrence of *Staphylococcus aureus* in fresh and fermented milk in Kaduna and Zaria. It was also lower than the 8.7% prevalence recorded by Okpo *et al.* (2016) in parts of Kaduna State, Nigeria. Higher rates of occurrence of *S. aureus* at 32%, 56%, 25.53%, and 55.26% were reported by Patrick *et al.* (2013), Gundogan & Avci (2014), Jahan *et al.* (2015), and Chaalal *et al.* (2016) in Kenya, Turkey, Bangladesh, and Algeria, respectively.

**Table 4:** Antibiotic susceptibility profile of *Staphylococcus aureus* isolated from fresh and fermented milk in parts of Nasarawa State.

| Antibiotics                     | Disc Conc. ( $\mu\text{g}$ ) | 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 5:** Antibiotic resistance pattern of *Staphylococcus 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 = Amoxillin/clavulanic acid, Fox = cefoxitin, Tet = Tetracycline, Ery= Erythromycin, Sul = Sulphamethoxazole/trimethoprim, NS = Nasarawa, KF = Keffi, AK = Akwanga, WM = Wamba, LF = Lafia, DM = Doma

This portrays *Staphylococcus aureus* as a bacterial pathogen of public health significance as it relates to food safety. 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 *Staphylococcus 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 are one of the major vehicles for the transmission of *S. aureus* to man (Jahan *et al.*, 2015). No significant difference ( $p > 0.05$ ) was found in the occurrence of *Staphylococcus 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 contrasted with previous report 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* (Kivaria *et al.*, 2006). 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 lactating cows, poor sanitary practices during milking, and unclean milking utensils (Kivaria *et al.*, 2006). 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 (Radostits *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 (Adams & Moss, 2011).

It should be noted that the use of water of unsatisfactory microbiological quality for milking and manufacturing of milk products is associated with the risk of contamination of milk with *S. aureus* (Kivaria *et al.*, 2006). The use of unclean water for washing the teat and udder of cow could lead to wet, dirty udder at milking time. Cross contamination can be avoided if the hands of milkers and milking utensils are washed adequately with detergent and clean water in between milking each cow and using the utensil. Hand washing is basic component of infection control (Larson *et al.*, 2003). Most (71.43%) of the containers used as 'bulk tanks' at the Fulani settlements were probably of unsatisfactory cleanliness which could have resulted in milk contamination with microorganisms. A significant association ( $p < 0.05$ ) was found between the kind of water used at the *nono* and *kindirmo* selling points and the occurrence of *Staphylococcus aureus* in the products. Majority of the *nono* and *kindirmo* selling points were using water purchased from vendors for washing utensils. Water contamination often occur in the storage containers and if the contaminated water gain access to milk or is used for rinsing containers, microorganisms present in the water will contaminate the milk (Kivaria *et al.*, 2006). The results indicate that the microbiological quality of milk product is influenced by factors associated with water quality. No significant association ( $p > 0.05$ ) was found between the occurrence of *Staphylococcus aureus* in *nono* and *kindirmo* and street activities (mainly vehicular movement) at the selling points, although the samples collected from the selling points with high street activities were found to be more contaminated compared to the samples collected from the selling points with low street activities. Many of the selling points were located in or close to motor parks, shops, and other non-dairy activities which put the products at a high risk of contamination.

The susceptibility of the isolates to gentamicin, ciprofloxacin, and chloramphenicol was in consonance with the findings of Okpo *et al.* (2016) and Rodrigues *et al.* (2017) in parts of Kaduna State, Nigeria and Brazil, respectively. The high performance of these antibiotics to 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 agrees with the assertion of Maillard (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 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 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 & Mustapha (2016), who reported 42.6 % and 66.7% resistance of *S. aureus* 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 (9) *S. aureus* isolates were resistant to ampicillin and amoxicillin/clavulanic acid. This could be attributed to the contamination of milk products (*nono* and *kindirmo*) with *S. aureus* strains that are resistant to  $\beta$ -lactam drugs derived from human sources. *S. aureus* resistant to one  $\beta$ -lactam drug can develop resistance to  $\beta$ -lactams because they have the same mechanism of activity (Suleiman *et al.*, 2012). 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 & 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. The relatively high frequency of resistance 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 &



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

Multidrug resistance is defined as resistance of an isolate to three or more antibiotics in different classes (Magiorakos *et al.*, 2011). This finding is in consonance with the findings of Umaru *et al.* (2013), Anueyiagu & 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 (3), four (4), five (5), and six (6) 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 (5) antibiotic resistance patterns among the *Staphylococcus aureus* isolates recorded in this study, varied with the nine (9) and 25 antibiotic resistance patterns recorded among *S. aureus* isolated from dairy products by Usman & Mustapha (2016), and Shiferaw & Ahmad (2016) in Kaduna State, Nigeria and Bahir Dar, Ethiopia, respectively. The disparity in the antibiotic resistance patterns of *Staphylococcus 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 different areas.

The detection of *Staphylococcus 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 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*. It is therefore recommended that since compliance with basic hygiene requirements is not guaranteed, hazard analysis and critical control points (HACCP) concepts should be seen as a part of an effective total hygiene concept at the selling points. There is need for frequent education of the *nono* and *kindirmo* sellers on the aspects of milk hygiene and handling practices, which will help in no small measure in improving the quality standards of the products at the selling points. This can be achieved through teaching and training programmes using participatory approach method. There is need for relevant authorities to educate the public on the

dangers of indiscriminate purchase and use of antibiotics.

#### Acknowledgement

We are grateful to the technical staff of Food Research Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria, and the technical staff of the Bacterial Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, for excellent technical assistance.

#### Conflicts of Interest

The authors declare no conflict of interest.

#### References

- Aarestrup FM (1995). Occurrence of glycopeptides resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Journal of Microbial Drug Resistance*, **25**(1): 255-257.
- Adams, MR & Moss MO (2011). *Food Microbiology (Reprinted Ed.)*. New Age International Publishers, New Delhi, India. Pp 104-209.
- Akram N, Chaudhary AH, Ahmed S, Ghuma MA, Nawaz G & Hussain S (2013). Isolation of bacteria from mastitis affected bovine milk and their antibiogram. *European Journal of Veterinary Medicine*, **2**(1): 38-46.
- Alian F, Rahimi E, Shakerian A, Momtaz H, Riahi M & Momeni M (2012). Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine, sheep, and goat raw milk. *Global Veterinaria*, **8**(2): 111-114.
- Anueyiagu KN & Isiyaku AW (2015). Isolation and identification of *Staphylococcus aureus* from bovine milk and its antibiotic susceptibility. *International Journal of Livestock Production*, **6**(6): 74-77.
- Baird-Parker AC (1962). An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. *Journal of Applied Bacteriology*, **25**(1): 10-12.
- Bianchi DM, Gallina S, Bellio A, Chiesa F, Civera F & Decastelli L (2014). Enterotoxin gene profiles of *Staphylococcus aureus* isolated from cow milk and dairy products in Italy. *Letters in Applied Microbiology*, **58**(2): 190-196.
- Chaalal W, Aggad H, Zidane K, Saidi H Kihal M (2016). Antimicrobial susceptibility profiling of *Staphylococcus aureus* isolates from milk.

*British Microbiology Research Journal*, **13**(3): 1-7.

- Chigor VN, Umoh VJ, Smith IS, Igbinosa OE & Okoh IA (2010). Multidrug resistance and plasmid patterns of *Escherichia coli* O157 and other *E. coli* isolated from diarrhoeal stools and surface waters from some selected sources in Zaria, Nigeria. *International Journal of Environmental Research and Public Health*, **7**: 3831-3841.
- Clinical and Laboratory Standards Institute (CLSI) (2016). Performance Standards for Antimicrobial Susceptibility Testing, twenty sixth edition. CLSI Supplement M100S. Wayne PA, USA. Pp 74-79.
- Esron D, Kariemuebo E, Lughano T, Kusiluka RH, Melegela AM, Kapaa M & Calvin S (2005). A study on mastitis, milk quality, and health risk associated with consumption of milk from pastoral herds in Dodoma, Morgora regions, Tanzania. *Journal of Veterinary Science*, **6**(3): 213-221.
- Gundogan N & Avci E (2014). Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *International Journal of Dairy Technology*, doi: 10.1111/1471-0307.12149.
- Jahan M, Marzia R, Shafiullah P, Shah ZHC, Emanul H, Abdul KT & Sultan A (2015). Isolation and characterisation of *Staphylococcus aureus* from raw cow milk in Bangladesh. *Journal. Advanced Animal Research*, **2**(1):49-55.
- Jamali H, Radmehrc B & Salmah I (2014). Short communication: Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Journal of Dairy Science*, **97**(4): 2226-2230.
- Jamali H, Paydarb M, Radmehrc B, Salmah I & Dadrasniaa A (2015). Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*, doi: 10.1016/j.foodcont.2015.02.013.
- Japoni A, Alborzi A, Rasouli M & Pourabbas B (2004). Modified DNA Extraction for rapid PCR detection of methicillin-resistant staphylococci. *Iranian Biomedical Journal*, **8**(3): 161-165.
- Junaidu AU, Salihu MD, Tambuwal FM, Magaji AA & Jaafaru S (2011). Prevalence of mastitis in lactating cows in some selected commercial

- dairy farms in Sokoto Metropolis. *Advances in Applied Science Research*, **2**(2): 290-294.
- Kadariya J, Smith TC & Thapaliya D (2014). *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *Biomedical Reserach. International*, doi.org/10.1111/lam.12182.
- Khachatourians G (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic resistant bacteria. *Canadian Medical Association Journal*, **159**(9): 1129-1136.
- Kivaria FM, Noordhuizen JPTM & Kapaga AM (2006). Evaluation of hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam Region, Tanzania. *Tropical Animal Health and Production*, **38**(3): 185-194.
- Korpysa-Dzirba W & Osek J (2011). Identification of genes encoding classical staphylococcal enterotoxins in *Staphylococcus aureus* isolated from raw milk. *Bulletin of Veterinary Institute Pulawy*, **55**(1): 55-58.
- Larson E, Aiello A, Lee LV, Della-Latta P, Gomez-Duarte C & Lin S (2003). Short and long term effects of hand washing with antimicrobial or plain soap in the community. *Journal of Community Health*, **28**(2): 139-50.
- Levin B, Lipsitch M, Pettot V, Schrag S, Anita R & Simonsen L (1997). The Population genetics of antibiotic resistance. *Journal of Clinical and Infectious Diseases*, doi: 10.1093/clinids/24.supplement\_1.s9.
- Maduka HCC, Ugwu CE, Maduka AA, Hashidu NH & Gimba BS (2013). Microbial screening and lipid peroxidation states of fermented milk (yoghurt) samples sold in Maiduguri metropolis and commonly-consumed in the University of Maiduguri, Borno State, Nigeria. *British Journal of Dairy Sciences*, **3**(2): 14-21.
- Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson, DL, Rice, LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT & Monnet DL (2011). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infectious Diseases*, **18**(3): 268-281.
- Maillard JY (2002). Bacterial target sites for biocide action. *Journal of Applied Microbiology*, doi: 10.1046/j.1365-2672.92.5s1.3.x.
- Mirzaei H, Farhoudi H, Tavassoli H, Farajli M & Monadi A (2012). Presence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* in raw and pasteurised milk and ice cream in Tabriz by culture and PCR techniques. *African Journal of Microbiology Research*, **6**(32):6224-6229.
- Naing L, Winn T & Rushi BN (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Science*, doi: 10.1.1.504.2129.
- Okpo NO, Abdullahi IO, Whong CMZ & Ameh JB (2016). Occurrence and antibiogram of *Staphylococcus aureus* in dairy products consumed in parts of Kaduna State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, **9**(2): 225-229.
- Olatoye IO (2010). The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *African Journal of Biotechnology*, **9**(8): 1196- 1199.
- Patrick MKN, Stefania D, Christophe J, John W, Christophe L & Leo M (2013). Phenotypic and genotypic antibiotic resistance patterns of *Staphylococcus aureus* from raw and spontaneously fermented camel milk. *British Journal of Science and Technology*, **3**(3): 87-98.
- Pereira V, Lopes C, Castro A, Silva J, Gibbs P & Teixeira P (2009). Characterization for enterotoxin production, virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiology*, **26**(3): 278-282.
- Peton V & Le Loir Y (2014). *Staphylococcus aureus* in veterinary medicine. *Infecton Genetics and Evolution*, doi: 10.1016/j.meegid.2013.08.011
- Phillips I, Casewell M, Cox T, De Groot B, Friis C & Jones R (2004). 'Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, **53**(1): 28-52.
- Radostits OM, Blood DC & Gay CC (1994). *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, eighth edition. Bailliere Tindall, London. Pp 563-613.

- Rodrigues MX, Silva NC, Trevilin JH, Cruzado MMB, Mui TS, Duarte FRS, Castillo CJC, Caniatti-Brazaca SG & Porto E (2017). Antibiotic resistance and molecular characterization of *Staphylococcus* species from mastitic milk. *African Journal of Microbiology Research*, **11**(3): 84-91.
- Shiferaw S & Ahmad M (2016). Prevalence and antibiotic susceptibility of *Staphylococcus aureus* from lactating cows' milk in Bahir Dar dairy farm, Ethiopia. *African Journal of Microbiology Research*, **10**(35): 1444-1454.
- Spanu V, Spanu C, Virdis S, Cossu F, Scarano C & de Santis EPL (2012). Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. *International Journal of Food Microbiology*, **153**(1-2): 53-57.
- Suleiman AB, Kwaga JKP, Umoh VJ, Okolocha EC, Muhammed M, Lammler C, Shaibu SJ, Akindele O & Weiss R (2012). Macro-restriction analysis of *Staphylococcus aureus* isolated from subclinical bovine mastitis in Nigeria. *African Journal of Microbiology Research*, **6**(33): 6270-6274.
- Tessema F (2016). Prevalence and drug resistance patterns of *Staphylococcus aureus* in lactating dairy cows' milk in Wolayta Sodo, Ethiopia. *EC Veterinary Science*, **2**(5): 226-230.
- Umaru GA, Kabir J, Umoh VJ, Bello M & Kwaga, JKP (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.
- Umoh VJ (1989). Contamination of *fura-da-nono* by staphylococci and growth of an enterotoxigenic *Staphylococcus aureus* in *fura*, a cereal food. *Zaria Veterinarian*, **4**(2): 53-58.
- Usman RZ & Mustapha BM (2016). Isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk 'nono' and yoghurt in Kaduna metropolis. *Food Science and Quality Management*, **2**(2): 1-21.
- Vázquez-Sánchez D, López-Cabo M, Saá-Ibusquiza P & Rodríguez-Herrera JJ (2012). Incidence and characterisation of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). *International Journal of Food Microbiology*, **157**(2): 286-296.
- Vyletělova M, Hanuš O, Karpišková R & Šťastková Z (2011). Occurrence and antimicrobial sensitivity in *staphylococci* isolated from goat, sheep and cow's milk. *Acta University of Agriculture et silvic Mendel Brun*, doi: 10.11118/actaun201159030209.
- Wayne PA (2002). Methods for dilution in antimicrobial susceptibility tests for bacteria that grow aerobically (fifth edition). Approved standard M7-A5. National Committee for Clinical Laboratory Standards (NCCLS), USA. Pp 10-23.
- Zhao S, White DG, Ge B, Ayers S, Friedman S, English L, Wagner D, Gaines S & J Meng J (2001). Identification and characterisation of integron-mediated antibiotic resistance among shiga toxin-producing *Escherichia coli* isolates. *Journal of Applied and Environmental Microbiology*, **67**(4): 1558-1564.