



Occurrence and Antibiogram of *Staphylococcus aureus* Isolated from Locally-Pasteurised Cow Milk (Kindirmo) Sold in Parts of Nasarawa Town, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all the authors. Author YA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CRR and AMS managed the analyses of the study. Author EMS managed the literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

This work was aimed at determining the occurrence and antibiogram of *Staphylococcus aureus* isolated from locally-pasteurised milk (*kindirmo*) sold in parts of Nasarawa Town, Nasarawa State, Nigeria. In this study, 123 samples were obtained from three different sampling points – Nasarawa Market, Tammah Area and Gunki Settlement. 12 samples yielded positive results for *S. aureus*, given an overall prevalence of 9.76%. Out of this positive samples, 6 positive samples were obtained from Nasarawa Market with a prevalence of 14.63%; 3 from Tammah Area with a prevalence of 7.32%, and 3 also were obtained from Gunki Settlement with a prevalence rate of 7.32%. Although 41 samples were collected from each of the three different sampling points, the

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highest prevalence was observed in samples collected from Nasarawa Market, (14.63%), with Tammah Area and Gunki Settlement having an equal prevalence rate of 7.32%. the reason for the disparity from the three sampling areas was not deciphered as the hawkers seemed to have subjected the milk to the same method of treatment (heating the fresh milk to a specific temperature for some period). The antibiotic susceptibility profile of the 12 *S. aureus* isolates revealed that the majority of the isolates were highly susceptible to ciprofloxacin and rifampicin. The isolates were observed to have developed high resistance to ampiclox, amoxicillin, streptomycin, chloramphenicol, erythromycin, norfloxacin and levofloxacin. 10 resistant phenotypes were observed with varying combinations of 2,3,4,5 and 6 antibiotics. All the isolates had multiple antibiotics resistant (MAR) index of 0.2 and above.

Keywords: *Staphylococcus aureus*; locally-pasteurised cow milk (kindirmo); antibiogram; Nasarawa town; Nigeria.

1. INTRODUCTION

Milk and milk products are essential components of the food of humans; source milk is one of the primary sources of nutrients especially for growing children. Milk is an excellent source of calcium, vitamins D, riboflavin, and phosphorous, and is also a good source of protein, potassium, vitamin A, vitamin B12 and niacin [1]. Despite the fundamental roles that milk and milk products play in human nutrition, they serve as vehicles for the transmission of many bacterial pathogens. The health risk to consumers can be associated with milk due to the presence of foodborne pathogens. Milk quality can be lowered by a number of factors such as contamination during and after milking, and the presence of adder infections. Pathogenic microorganisms in milk can be derived from the cow itself, the human hand, or the environment [2,3].

Raw milk and various types of fermented milk are produced and consumed as supplements to regular meals in homes and even for sale [4]. Many families consumed raw milk simply because it is a traditional practice and less expensive to buying pasteurised retailed milk [5]. Raw milk other traditional dairy products such as *nono* (locally-fermented milk) are commonly hawked and consumed on many northern Nigerian streets [6].

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 [7]. Milk is considered as a good substrate on which *S. aureus* grow and produce enterotoxins [8]. Some of these milk products due to their processing and distribution practice are exposed to conditions which may permit

growth of contaminating organisms and possible toxin producers [9].

Mastitis caused by *S. aureus* is a serious problem in dairy production and infect animals may contaminate the bulk milk. In addition, human handlers, milking equipment the environment and udder and test skin of dairy animals are other likely sources of bulk milk contamination. Food handlers carrying enterotoxigenic *S. aureus* in theirs or in orifices such as the nose, are regarded as the major source of food contamination, via contact or respiratory secretions, contamination is mainly associated with improper handling of fresh, cooked or processed foods, followed by storage under conditions which allow the growth of *S. aureus* and production of enterotoxins. Air, dust, and food contact surfaces can also serve as vehicles for the transmission of *S. aureus* [10].

From the perspective of food safety, this is a cause for concern because enterotoxigenic strains of *S. aureus* poses a risk of SFP when contaminated milk and milk products are consumed [11]. Antibiotic usage has become commonplace in human medicine and animal production. The extensive use of antibiotic in both human 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 [12]. The development of resistance to antibiotics is known to occur through stable genetic exchange heritable from generation to generation through specific mechanism namely, transduction, transformation and/or conjugation [13].

S. aureus is associated with a variety of clinical infections involving septicaemia, pneumonia,

wound sepsis, septic arthritis, osteomyelitis with substantial morbidity and mortality [14]. One of the reasons for the success of this pathogen is its great variability occurring at different periods and places with diverse clonal types and antibiotic resistance patterns within regions and countries [15].

Pathogenic strains of *S. aureus* are usually coagulase-positive and cause disease in their hosts. The manifestation of the infection range from abscesses or mastitis to a severe toxic shock syndrome. The bacteria contaminates milk during milking and the contamination depends on the sanitary condition of the plant, milking utensils and milking personnel. Contamination may also result from the microorganisms entering the udder through teat canal [16].

The hygienic standard practices in a milk plant may be evaluated based on the level of contamination with *S. aureus* and the assessment of the antibiotic-resistant phenotypes serves as a tool for evaluating the hygienic standards adhered to during milking. Antibiotic sensitivity pattern of the isolates are also useful in characterising this pathogen. The characterisation may help reduce the risks of infection through milk and milk products [17]. Considering the aforementioned public health concerns, this research work was aimed at isolating and characterising *S. aureus* from locally-pasteurised cow milk (*kindirmo*), determining the antibiotic susceptibility profiles, the antibiotic resistance patterns, and the multiple antibiotics' resistance (MAR) indices of the *S. aureus* isolates.

2. MATERIALS AND METHODS

2.1 The Study Area

This study was carried out in Nasarawa town. Nasarawa is a town in Nasarawa state in the Northern part of Nigeria [18]. It has an area of about 5,740 km² with a population of 189,835 as at the 2006 census as documented by the National Bureau of statistics [18].

It is approximately 105 km from Abuja, the Federal Capital Territory, 37km from Keffi and 165 km from Lafia, the state capital. The town is located between latitude 8°21'58"N of the equator between latitude 7°5'58E of the Greenwich meridian as recorded by the National Bureau of Statistics [18].

2.2 The Sampling Design and Techniques

2.2.1 The sample size

The sample size was determined by the use of prevalence of 8.75% as reported by [19]. The sample size was determined by using equation described by [20].

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

Where:

n -Is the sample size

P – Is the prevalence from a previous study = 8.75% = 0.0875.

Z – Is the standard normal distribution at 95% confidence interval = 1.96.

D – is the absolute desired precision at 5% = 0.05.

$$\text{Therefore, } n = \frac{(1.96)^2 \times 0.08750 (1-0.08750)}{(0.05)^2}$$

$$= \frac{0.30672775}{0.0025}$$

$$= 122.69 = 123 \text{ samples}$$

2.2.2 Sample collection

A total number of 123 samples of locally-pasteurised milk (*kindirmo*) were collected from four different retail points in Nasarawa Towns namely: Nasarawa Market, Tammah Area, and Gunki settlement. The samples were transported aseptically wrapped in polythene bags and transported to the Microbiology Laboratory of the Federal Polytechnic, Nasarawa and analysed immediately.

2.3 Isolation and Identification of *S. aureus* from Milk Samples

The collected milk samples (0.01 mL) were streaked onto prepared plates of Mannitol salt agar (MSA) (HiMedia®, India) and incubated at 37°C for 24 h. The presumptive colonies of *S. aureus* were further sub-cultured onto mannitol salt agar (MSA) and repeatedly sub-cultured order to get pure culture. These isolates were preserved for further bacterial identification. The isolates were identified as *S. aureus* on the basis of Gram staining, colony morphology on mannitol salt agar (MSA) (HiMedia®, India), beta-hemolytic patterns on blood agar enriched with

5% (v/v) sheep blood, catalase test, DNase test, and coagulase tests [21]. To perform agglutination tests, the pure colony of *S. aureus* were placed on the clean glass slide using sterile inoculation loop and a drop of respective reagents was added and mixed with the loop. For catalase and coagulase tests 3% hydrogen peroxide and fresh rabbit plasma were used respectively. For the DNase test, suspected colonies of *Staphylococcus aureus* were inoculated onto prepared DNase agar plates. The plates were incubated overnight at 37°C. Presence of DNase was tested by flooding plates with a weak 1N HCl. Polymerised DNA precipitated in the presence of 1N HCl gives an opaque medium. A positive organism shows clear zones around colonies [21].

2.4 Antibiotic Susceptibility Test of the Isolates

All the *S. aureus* isolates were subjected to antibiotic sensitivity testing by standard agar disc diffusion method on Muller-Hinton agar (Oxoid, UK) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [22]. A MacFarland 0.5 standardised suspension of the bacterium (1.5×10^8 cells/ml) in 0.8% sterile saline was prepared and swabbed over the entire surface of Mueller-Hinton agar (Oxoid) with a sterile cotton swab [23]. Sensitivity pattern of the isolates to: ciprofloxacin (10 µg), (gentamicin (10 µg), streptomycin (30 µg), norfloxacin (10 µg), chloramphenicol (30 µg), amoxicillin (20 µg), erythromycin 30 µg), ampiclox (20 µg), and levofloxacin (20 µg) (Oxoid) and rifampicin (20 µg) was evaluated (Antimicrobial Susceptibility Test System, UK). After a 24-hour incubation at 37°C, clear zones produced by antibiotic inhibition of the bacterial growth were measured in millimetre (mm) using a straight transparent ruler on the underside of the plate. The results were interpreted using the Clinical Laboratory Standards Institute (CLSI) criteria (CLSI, 2008). Results obtained for each isolate were interpreted as 1). Sensitive (S): if the observed zone of the inhibition diameter was equal or greater than CLSI sensitive diameter; 2). Intermediate (I): if the observed zone of inhibition diameter falling within the intermediate range between the CLSI resistant and sensitive limits; 3). Resistant (R): if the observed zone of inhibition diameter was less than or equal to the CLSI resistant diameter according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [22].

2.5 Determination of the Multiple Antibiotics Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate (s) is resistant, by the total number of antibiotics tested

MAR Index = (Number of antibiotics to which the isolate is resistant/ Total number of antibiotics tested) [24]

3. RESULTS

Table 1 shows the prevalence (%) of *S. aureus* isolates obtained from the three different sampling points selected for this study. Of the 123 samples collected in this study, 12 yielded positive results for *S. aureus* giving an overall prevalence of 9.76%. Out of the 41 samples collected from Nasarawa Market, 6 yielded positive results for *S. aureus* with a prevalence of 14.63%; of the 41 samples collected from Tammah Area, 3 yielded positive results for *S. aureus* with a prevalence of 7.32%; and of the 41 samples collected from Gunki settlement, 3 yielded positive results for *S. aureus*, giving a prevalence of 7.32%.

Table 2 shows the antibiotic susceptibility profile of *S. aureus* isolates obtained from locally-pasteurised milk sold in Nasarawa Market. While 5 (83.3%) were resistant to ampiclox, 1 (16.7%) exhibited intermediate susceptibility to the same antibiotic. None of the isolates was susceptible to the antibiotic. 4 (66.7%) of the isolates were resistant to streptomycin, while 2 (33.3%) exhibited intermediate susceptibility to the same antibiotic. 5 (83.3%) of the isolates were resistant to norfloxacin, while 1 (18.7%) was susceptible to the same antibiotic. 6 (100%) of the isolates were susceptible to chloramphenicol and gentamicin, while 1 (16.7%) of the isolates exhibited intermediate susceptibility and 2 (33.3%) exhibited resistance to the same antibiotics. 1 (16.7%) was susceptible to rifampicin and 5 (83.3%) were resistant to the same antibiotic. 2 (33.3%) were susceptible to ciprofloxacin and erythromycin, while 4 (66.7%) exhibited intermediate susceptibility to the same antibiotics. 3 (50%) of the isolates were resistant to levofloxacin, while 3 (50%) exhibited intermediate susceptibility to the same antibiotic (Table 2).

Table 3 shows the antibiotic susceptibility profile of *S. aureus* isolates obtained from locally-

pasteurised milk sold in Tammah. Of the 3 isolates obtained from samples collected from this area, 2 (66.7%) were susceptible to ciprofloxacin and gentamicin, while 1 (33.3%) exhibited intermediate susceptibility to the antibiotic (gentamicin). All the 3 (100%) of the isolates exhibited intermediate susceptibility to norfloxacin. All the 3 (100%) of the isolates were resistant to amoxicillin. 1 (33.3%) of the isolates were susceptible to streptomycin and rifampicin, while the remaining 2 (66.7%) exhibited intermediate susceptibility to the antibiotic (streptomycin). All the 3 (100%) of the isolates obtained from samples collected from this area exhibited intermediate susceptibility to erythromycin, chloramphenicol and levofloxacin. 1 (33.3%) of the isolates exhibited intermediate susceptibility to ampiclox, while the remaining 2 (66.7%) showed complete resistance to the antibiotic (Table 3).

Table 4 shows the antibiotic susceptibility of *S. aureus* isolates obtained from locally-pasteurised milk sold in Gunki settlement. All of the 3 (100%) isolates obtained from the samples were susceptible to ciprofloxacin, 2 (66.7%) of the isolates showed susceptibility to gentamicin and norfloxacin, and 1 (33.3%) exhibited intermediate susceptibility to the antibiotics (gentamicin and norfloxacin). All the 3 (100%) of the isolates exhibited intermediate susceptibility to streptomycin and rifampicin; 2 (66.7%) of the isolates exhibited intermediate susceptibility to erythromycin and chloramphenicol, while the remaining 1 (33.3%) was resistant to the antibiotics. 1 (33.3%) of the isolates was susceptible to levofloxacin, while the remaining 2 (66.7%) were resistant to levofloxacin. All the 3 (100%) obtained from samples collected in this area showed resistance to ampiclox (Table 4).

Table 5 shows the antibiotic resistance patterns of *S. aureus* isolated from locally-pasteurised milk sold in parts of Nasarawa Town. 10 resistant phenotypes were obtained, all with multiple resistant types with a varying combination of 2,3,4,5 and 6 antibiotics. No resistant phenotype was found with a single

antibiotic. The highest frequency (4) (of isolates showing resistance to a combination of antibiotics) was found in the combination with 4 antibiotics. The multiple antibiotic resistance (MAR) index of *S. aureus* isolates is as shown in Fig. 1.

4. DISCUSSION

Of the one hundred and twenty-three (123) samples collected for this study, the prevalence of *S. aureus* isolates obtained from the three different sampling points – Nasarawa Market, Tammah Area, and Gunki Settlement showed that 12 yielded a positive result for *S. aureus* giving an overall prevalence of 9.76%. The prevalence recorded in this study was higher than the 4.8% prevalence recorded by [25] in a study carried out to isolate methicillin-resistant *S. aureus* in fresh and fermented milk in Zaria and Kaduna State. The paucity of information on *S. aureus* isolated from locally-pasteurised milk and other foods in Nasarawa town make it difficult to make any comparison and to assess *S. aureus* status in milk in Nasarawa town. Out of the 41 samples collected from Nasarawa market, 6 yield positive results for *S. aureus* with a prevalence of 4.63%; out of the 41 samples collected from Gunki Settlement, 3 yielded positive results for *S. aureus* with a prevalence of 7.32%; and out of the 41 samples collected from Tammah Area, 3 yielded positive results for *S. aureus*, giving a prevalence of 7.32%.

The occurrence of *S. aureus* (9.76%) in locally-pasteurised milk (*kindirmo*) consumed in these locations poses a serious public health threat for consumers of these local dairy products, particularly as the transfer of pathogens via such food chain is very well documented. *Kindirmo* is prepared by boiling whole fresh milk for about 20 min. The boiled whole fresh milk is then allowed to cool and ferment overnight spontaneously in a local calabash. After fermentation, water is added to the product to dilute it thus increasing its visible quantity. The occurrence might be

Table 1. Prevalence (%) of *S. aureus* obtained from locally-pasteurised milk (Kindirmo) sold in parts of Nasarawa town

| Sampling point | No. collected | No. positive | Prevalence (%) |
|----------------|---------------|--------------|----------------|
| NM | 41 | 6 | 14.63 |
| TM | 41 | 3 | 7.32 |
| GK | 41 | 3 | 7.32 |
| Total | 123 | 12 | 9.76 |

Key: NM – Nasarawa Market; TM – Tammah Area; GK – Gunki Settlement

Table 2. The antibiotic susceptibility profile of *S. aureus* isolated from locally-pasteurised milk sold in Nasarawa market

| Antibiotics | Disc Conc. (μg) | (n=6) | | |
|-----------------|------------------------------|---------|---------|----------|
| | | S (%) | I (%) | R (%) |
| Ciprofloxacin | (Cip, 10 μg) | 2(33.3) | 4(66.7) | 0(0.0) |
| Norfloxacin | (Nor, 10 μg) | 1(16.7) | 0(0.0) | 5(83.3) |
| Gentamicin | (Gen, 10 μg) | 3(50.0) | 1(16.7) | 2(33.3) |
| Amoxil | (Amo, 20 μg) | 0(0.0) | 0(0.0) | 6(100.0) |
| Streptomycin | (Str, 30 μg) | 0(0.0) | 2(33.3) | 4(66.7) |
| Rifampicin | (Rif, 20 μg) | 1(16.7) | 0(0.0) | 5(83.3) |
| Erythromycin | (Ery, 30 μg) | 2(33.3) | 4(66.7) | 0(0.0) |
| Chloramphenicol | (Chl, 30 μg) | 3(50.0) | 1(16.7) | 2(33.3) |
| Ampiclox | (Amp, 20 μg) | 0(0.0) | 1(16.7) | 5(83.3) |
| Levofloxacin | (Lev, 20 μg) | 0(0.0) | 3(50.0) | 3(50.0) |

Key: Susceptible, I = Intermediate, R = Resistant

Table 3. The antibiotic susceptibility profile of *S. aureus* isolates obtained from locally-pasteurised milk sold in Tammah area

| Antibiotics | Disc Conc. | (n=3) | | |
|-----------------|--------------------------|---------|----------|----------|
| | | S (%) | I (%) | R (%) |
| Ciprofloxacin | (Cip, 10 μg) | 2(66.7) | 1(33.3) | 0(0.0) |
| Norfloxacin | (Nor, 10 μg) | 0(0.0) | 3(100.0) | 0(0.0) |
| Gentamicin | (Gen, 10 μg) | 2(66.7) | 1(33.3) | 0(0.0) |
| Amoxil | (Amo, 20 μg) | 0(0.0) | 0(0.0) | 3(100.0) |
| Streptomycin | (Str, 30 μg) | 1(33.3) | 2(66.7) | 0(0.0) |
| Rifampicin | (Rif, 20 μg) | 1(33.3) | 2(66.7) | 0(0.0) |
| Erythromycin | (Ery, 30 μg) | 0(0.0) | 3(100.0) | 0(0.0) |
| Chloramphenicol | (Chl, 30 μg) | 0(0.0) | 3(100.0) | 0(0.0) |
| Ampiclox | (Amp, 20 μg) | 0(0.0) | 1(33.3) | 2(66.7) |
| Levofloxacin | (Lev, 20 μg) | 0(0.0) | 3(100.0) | 0(0.0) |

Table 4. The antibiotic susceptibility profile of *S. aureus* isolates obtained from locally-pasteurised milk sold in Gunki settlement

| Antibiotics | Disc Conc. | (n=3) | | |
|-----------------|--------------------------|----------|----------|----------|
| | | S (%) | I (%) | R (%) |
| Ciprofloxacin | (Cip, 10 μg) | 3(100.0) | 0(0.0) | 0(0.0) |
| Norfloxacin | (Nor, 10 μg) | 2(66.7) | 1(33.3) | 0(0.0) |
| Gentamicin | (Gen, 10 μg) | 2(66.7) | 1(33.3) | 0(0.0) |
| Amoxil | (Amo, 20 μg) | 0(0.0) | 0(0.0) | 3(100.0) |
| Streptomycin | (Str, 30 μg) | 1(33.3) | 0(0.0) | 2(66.7) |
| Rifampicin | (Rif, 20 μg) | 0(0.0) | 3(100.0) | 0(0.0) |
| Erythromycin | (Ery, 30 μg) | 0(0.0) | 3(100.0) | 0(0.0) |
| Chloramphenicol | (Chl, 30 μg) | 0(0.0) | 2(66.7) | 1(33.3) |
| Ampiclox | (Amp, 20 μg) | 0(0.0) | 0(0.0) | 3(100.0) |
| Levofloxacin | (Lev, 20 μg) | 1(33.3) | 0(0.0) | 2(66.7) |

attributed to improper public health measures, sanitary and poor hygiene of people concerned with milk handling. Studies among the 3 sampling areas revealed that the percentage prevalence rate in Nasarawa main market area was 14. 63%. This may be as a result of the negligence for good environmental sanitation

such as hand washing with soap or hand sanitiser before, during, and after food handling. The reason for disparity from the three sampling area is not clearly elucidated as the hawkers seemed to have subjected the milk to the same method of treatment (heating the fresh milk to a certain temperature for

some period of time). The prevalence of *S. aureus* in raw milk and the dairy product was found to be 56% in Turkey by [26] and 75% in Bangladesh by [27] which were significantly higher than the one recorded in the present study.

Table 5. The antibiotic resistance patterns of *S. aureus* isolates obtained from locally-pasteurised milk (*Kindirmo*) sold in parts of Nasarawa Town

| No. of antibiotics | Resistance pattern | No. of isolates |
|--------------------|------------------------------|-----------------|
| 2 | Nor, Amo | 1(8.3) |
| 2 | Amo, Str | 1(8.3) |
| 3 | Amo, Amp, Lev | 1(8.3) |
| 4 | Amo, Chl, Amp, Lev | 2(16.7) |
| 4 | Nor, Amo, Rif, Amp | 2(16.7) |
| 4 | Amo, Str, Rif, Amp | 1(8.3) |
| 4 | Amo, Str, Ery, Amp | 1(8.3) |
| 5 | Gen, Amo, Chl, Amp, Lev | 1(8.3) |
| 5 | Nor, Amo, Str, Rif, Amp | 1(8.3) |
| 6 | Nor, Gen, Amo, Rif, Amp, Lev | 1(8.3) |

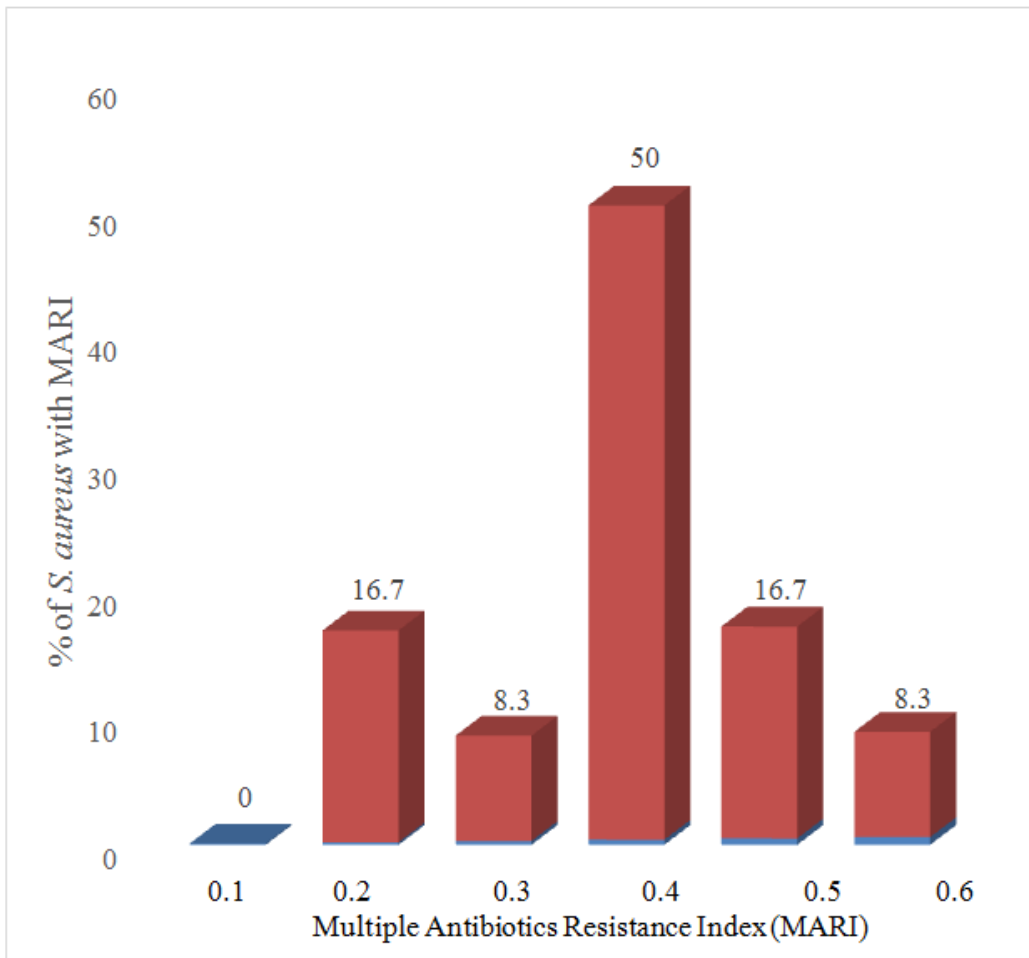


Fig. 1. Multiple antibiotics resistance (MAR) Index of *S. aureus* isolated from locally pasteurised milk sold in parts of nasarawa town (100% of the *S. aureus* isolated had MAR index of 0.2 and above)

Based on the antibiotic susceptibility profile of the 12 *S. aureus* isolates out of the 123 samples obtained from locally-pasteurised milk sold in Nasarawa Market, Tammah Area and Gunki Settlement revealed that most of the isolate was found to be multidrug resistant. The isolate were found to be 100% resistant to ampiclox and amoxil, 66.7% were found to be resistant streptomycin, 33.3% were resistant to chloramphenicol, gentamicin, 83.3% were resistant to norfloxacin and levofloxacin. This is somewhat similar to the findings recorded by [28] and [29] where they analysed 119 isolates of *S. aureus* collected between 1998 and 2000 in France from cows with clinical mastitis. In another study in Bangladesh conducted by [27] revealed that *S. aureus* was 82.86% and 37.14% resistant to penicillin-G and amoxicillin, respectively; however, in this study it was observed that *S. aureus* showed complete resistance to amoxicillin and ampiclox, indicating the increasing resistance of the organism against amoxicillin. These findings are in line with the study carried out by [30] and this may be because they belong to the same family as penicillin, a β -lactam group of antibiotics and also exhibit the same mechanism of action. Similar resistance pattern was also reported by [31,32]. The high level of resistance of the isolates to amoxicillin and ampiclox may be as a result of use or misuse of the antibiotics in animal husbandry.

The results of this study indicate that ciprofloxacin and erythromycin stood out to be the drugs of choice for the treatment of *S. aureus* infection in this locality since none of the isolates showed resistance to them. The demonstration of high susceptibility to these antibiotics may have implications on the effectiveness of the local and probably antibiotics resistance control programmes. The high performance of erythromycin could also be due to their molecular sizes, a factor which enhances their solubility in diluents, thus, promoting their penetration power through cell wall to its target site (the bacterial ribosome) where it binds to the P-site of the 50 S subunit of the bacterial ribosome to inhibit peptide chain elongation during protein synthesis. This finding is in line with that of [33] who opined that the high efficacy of the antibiotics may be traced to their molecular sizes.

Staphylococcus aureus isolates obtained from locally-pasteurised milk samples in this study were observed to have exhibited multiple resistance to the antibiotics tested. On the whole,

10 resistance phenotypes were observed with varying combinations of 2,3,4,5, and 6 antibiotics. No resistance phenotype was found with a single antibiotic. The highest frequency (4) (of isolates showing resistance to a combination of antibiotics) was found in the combination with 4 antibiotics. This is in contrast with the findings of [30] who recorded 9 resistance phenotypes among methicillin-resistant *Staphylococcus aureus* isolated from traditionally fermented milk (*nono*) and yoghurt in Kaduna Metropolis, Nigeria. One hundred percent (100%) of the *S. aureus* isolates in this study were found to have had multiple antibiotic resistance indexes (MARI) of 0.2 and above. MAR index was calculated as the ratio of the number of antibiotics to which an organism is resistant to a total number of antibiotics to which an organism is exposed [34].

MAR index gives an indirect indication of the probable source of an organism. MAR index greater than 0.2 suggests there are no strict regulations concerning prescription and usage of antibiotics [24].

Residues of antibiotics have also been reported in tissues of food animals and their products [35,36]. The high percent occurrence of multiple antibiotic resistance (MAR) index (100%) among the *S. aureus* isolates in this study might have arisen due to common practices such as self-medication and the over-the-counter usage of antibiotics. In addition to this is the continued administration of antibiotics against infections that appear non-responsive to the normal dose given earlier [37].

5. CONCLUSION

To this end, the result obtained in this study revealed that the locally-pasteurised milk (*kindirmo*) sold in parts of Nasarawa town might be contaminated with *Staphylococcus aureus*. The presence of this pathogen in the product could be attributed to poor hygienic practices during production, packaging, and distribution. Samples obtained from Nasarawa market were found to have had the highest prevalence rate (14.63%) of *S. aureus*. The occurrence of *S. aureus* (9.76%) in locally-pasteurised milk consumed in the study areas poses a serious public health threat to consumers of the product primarily as the transmission of pathogens via the food chain is well documented. It is evident in this study that *S. aureus* isolates exhibit resistance to streptomycin, chloramphenicol, gentamicin, norfloxacin and levofloxacin. High

(100%) levels of antimicrobial resistance to antibiotics such as ampiclox and amoxil used were also observed. Apparently, isolate from the 3 sampling areas were found to be highly susceptible to ciprofloxacin, erythromycin and rifampicin, making them the drugs of choice for the treatment of *S. aureus* in this locality since none of the isolates showed resistance to them. Findings in this study suggest that *kindirmo* serves as a vehicle for the transmission of food-borne pathogens and is a food safety concern. This study also observed that *S. aureus* is becoming increasingly resistant to antimicrobial agents that are commonly employed in human and animal medicine.

6. RECOMMENDATIONS

1. There is need to put in place, effective control measures to safeguard public health from this foodborne pathogens. This involves making legislation that would mandate milk hawkers to pasteurise their products.
2. Milk hawkers should be enlightening and educated on how to maintain adequate hygiene during production, handling and distribution of their product. This can achieve through workshops seminars, herd and farms visit by the relevant authorities.
3. There is need to include different antibiotics in the panel during susceptibility testing of *S. aureus* in order to arrive at the best choices of treatment of *S. aureus* infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. United States Department of Agriculture (USDA). Dietary guidelines for Americans (6th ed.); 2005. Available:www.healthoerus.gov/dietaryguidelines.org
2. Bradely JA. Bovine mastitis – an evolving disease. Vet. J. 2002;164:116-128.
3. Esron D, Kariemuebo E, Lughano T, Kusiluka RH, Melegela AM, Kapaa M, Calvin S. 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. 2005;6: 213-221.
4. Maduka HCC, Ugwu CE, Maduka AA, Hashidu NH, Gimba BS. Microbial screening and lipid peroxidation status of fermented (Yoghurt) milk samples sold in Maiduguri metropolis and commonly consumed In University of Maiduguri Borno State, Nigeria. British Journal of Dairy Sciences. 2013;3(3):14-21.
5. Hegarty HO, Sullivan MB, Buckley J, Foley N. Continued raw milk consumption of farms; why? Community Discuss. Public Health. 2002;5:151-156.
6. Umoh VJ, Adesiyun AA, Gomwalk NE. Enterotoxigenicity of staphylococci isolated from raw milk obtained from Settled and nomadic herds around Zaria, Nigeria. Journal of Veterinary Medicine. 1990; 43(1):43-47.
7. Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, Chien MS, Lin TY. The emergence of fluoroquinolone-resistance in *Salmonella enterica* subtype *Choleraesius*. New England Journal of Medicine. 2002;346:413-419.
8. Korpysa-Dzirba W, Osek J. Identification of genes encoding classical *staphylococcal enterotoxins* in *Staphylococcus aureus* isolated from raw milk. Bull. Vet. Inst. Pulawy. 2011;55:55-58.
9. Ezeonu IM, Ezurike OA. Isolation and characterization of enterotoxigenic *Staphylococcus aureus* from Yoghurt samples. In: Igbinosa IB (Ed.). Annals of Natural Sciences. 2007;7(1):1-12.
10. Argudin MA, Mndoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2010;2:1751-1773.
11. Jorgensen HJ, Mark T, Hogasen HR, Rovik LM. Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. Journal of Applied Microbiology. 2005;99:158-167.
12. Callaway RT, Anderson CR, Elder RO, Edrington ST, Genovese JK, Bischoff MK, Poole LT, Jung SY, Harvey BR, Nisbet JD. Pre-slaughter intervention strategies to reduce food-borne pathogens in food animals. Journals for Animals Science. 2003;81:2.
13. Goodman AG, Theodore WR, Alan SN, Palmer T. The Pharmacological Basis of Therapeutics (8th Edition). Pergamoon Press. 1990;1020-1021.
14. Eneemann JJ, Carmel Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL,

- Briggs JP, Sexton DJ, Kaye KS. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* Surgical Sites Infection. *Clinical Infection Diseases*. 2003;36:592-598.
15. Akindede AA, Adewuyi IK, Adefioye OA, Adedokun SA, Oladu AO. Antibigram and beta-lactamase production of *Staphylococcus aureus* isolates from different human clinical specimens in tertiary health institute in Ile-Ife, Nigeria. *American Eurasian J. Sci Res*. 2010;5(4):230-233.
 16. Smith K, Peter K, Daniela H, Melchior S. Food borne pathogenic microorganisms and natural toxins. *Food Drug Administration Centre Food Safety, Applied Nutrition*. 2007;10:119-150.
 17. Wubete Y. Bacteriological quality of bovine milk in small holder dairy farms in Debre-Zeit, Ethiopia. M.Sc Thesis, Addis Ababa University; 2009.
 18. National Bureau of Statistics. Federal Republic of Nigeria, 2006 Population Census; 2009. Available:<http://nigeriastat.gov.ng/connection/pop2006.pdf>
 19. Okpo NO, Abdullahi IO, Whong CMZ, Ameh JB. Occurrence and antibiogram of *Staphylococcus aureus* in dairy products consumed in parts of Kaduna State, Nigeria. *Bayero Journal of Pure and Applied Sciences*. 2016;9(2):225-229.
 20. Naing L, Winn T, Rushi BN. Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Science*. 2006;1:9-14.
 21. Japoni A, Alborzi A, Rasouli M, Pourabbas B. Modified DNA extraction for rapid PCR detection of methicillin resistant staphylococci. *Iran Biomed J*. 2004;8(3): 161-165.
 22. Clinical Laboratory Standards Institute, CLSI. Performance standards for antimicrobial susceptibility testing. Eighteen Information Supplement. 2008;28(1):34-52.
 23. Coyle MB. Manual of antimicrobial susceptibility testing. American Society of Microbiology Press, Washington D.C. 2005;25:39.
 24. Olayinka BO, Olonitola OS, Olayinka AT, Agada EA. Antibiotic susceptibility pattern and multiple antibiotics resistance index of *P. aeruginosa* isolates from a university teaching hospital. *African Journal of Clinical and Experimental Microbiology*. 2004;5(2):198-200.
 25. Umaru GA, Kabir J, Umoh VJ, Bello M, Kwaga JKP. Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *International Journal of Drug Research and Technology*. 2013;3(3):67-75.
 26. Gundoga N, Avci E. Occurrence of antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw dairy products in Turkey. *International Journal of Dairy Technology*; 2014. DOI: 10.1111/1471-0307.12149
 27. Begum HA, Uddin MS, Islam MJ, Nazir KHMNH, Islam MA, Islam MT. Detection of biofilm producing coagulase positive *S. aureus* from Bovine Mastitis, their pigment production, haemolytic activity and antibiotic sensitivity pattern. *Journal of Bangladesh Society for Agricultural Science and Technology*. 2007;4:97-100.
 28. De Oliveira AP, Watts JL, Salmon SA, Aarestrup FM. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United states. *Journals of Dairy Science*. 2000; 83:855-862.
 29. Guerin FV, Carret G, Houffstschmitt P. *In vitro* activity of 10 antimicrobial agents against bacteria isolated from cows with clinical mastitis. *Veterinary Record*. 2003; 152:466-471.
 30. Usman RZ, Mustapha BM. Isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in traditionally-fermented milk 'nono' and yoghurt in Kaduna Metropolis, Nigeria. *Food Science and Quality Management*. 2016;55:42-50.
 31. Islam MJ, Uddin MS, Islam MA, Nazir KHMNH, Rahman MT, Alam MM. Prevalence of enterotoxigenic and toxic shock syndrome toxin-1 producing coagulase positive *Staphylococcus aureus* in human and their characterisation. *Bangladesh Journal of Veterinary Medicine*. 2007a;5:115-119.
 32. Islam MJ, Uddin MS, Islam MA, Nazir KHMNH, Rahman MT, Alam MM. Detection and characterisation of enterotoxins and toxic shock syndrome toxin -1 producing coagulase positive *Staphylococcus aureus* from bovine origin.

- The Bangladesh Veterinarian. 2007b;24: 27-23.
33. Mailard JY. Bacterial target sites for biocide action. Journal of Applied Microbiology. 2002;82:53-60.
34. Furtula V, Jackson CR, Farell EG, Barrett JB, Hiott LM, Chambers PA. Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. International Journal of Environmental Research and Public Health. 2013;10:1020-1036.
35. Kabir J, Umoh VJ, Audu-Okoh E, Umoh JU, Kwaga JKP. Veterinary drug use in poultry farms and determination of antimicrobial drug residue in commercial eggs and slaughtered chickens in Kaduna State, Nigeria. Food Controls. 2004;15:99-105.
36. Adesokan HK, Agada CA, Adetunji VO, Akanbi IM. Oxytetracycline and penicillin – G residues in cattle slaughtered in South-western Nigeria: Implications for livestock diseases management and public health. Journal of the South African Veterinary Association. 2013;84(1):945:1-5.
37. Ezenduka EV, Obegbulem ST, Nwanta JA, Andonukwo JI. Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. Tropical Animal Health and Production. 2011;43(3):557:559.

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