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# ANTIBIOGRAM OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) STRAINS FROM *KINDIRMO* IN NASARAWA, NASARAWA STATE, NIGERIA

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## ABSTRACT

Dairy products have been found to be a major vehicle for the transmission of multidrug-resistant MRSA strains to man. This study determined the antibiogram of methicillin-resistant *Staphylococcus aureus (*MRSA) strains from traditionally-pasteurized dairy product (*Kindirmo*) in selected parts of Nasarawa, Nasarawa State, Nigeria. One hundred and sixty *kindirmo* samples were collected from vendors using random sampling from the areas selected for the study. Sample collection was between January 2021 and April 2021. Each sample was collected into sterile screwed-capped plastic bottle and labeled appropriately. Standard microbiological procedures were used in isolating and identifying MRSA strains from the samples. Characterisation of the MRSA strains was carried out using Microgen® kits. The MRSA strains were evaluated for their susceptibility to cefoxitin (30µg), clindamycin (2µg), chloramphenicol (30µg), doxycycline (30µg), gentamicin (10µg), sulphamethoxazole/trimethoprim (25µg), tobramycin (30µg), and vancomycin (30µg), using the Kirby-Bauer technique. Of the 160 samples examined, eight MRSA strains were obtained, giving a prevalence of 5.0%. All of the MRSA strains were resistant to cefoxitin (0-1mm); 62.5% were resistant to tobramycin (7-11mm); and 25.0% were resistant to chloramphenicol (4-10mm). Five (5) antibiotic resistant phenotypes were recorded among the MRSA strains. The occurrence of MRSA in *Kindirmo* as recorded in this study, suggest that, the consumption of the product constitute a hazard to consumers. Basic hygiene requirements during production and selling of the product should be imposed by relevant authorities. This will go a long way in ensuring the safety of the product.

**Keywords**: Antibiogram, MRSA, Dairy Product, Nasarawa, Nigeria

## INTRODUCTION

Traditionally produced dairy products made under unhygienic conditions, are possible channels through which foodborne pathogens such as *Staphylococcus aureus* can be transmitted to man (Kadariya *et al*., 2014). *Staphylococcus aureus*, including those found in livestock, have been frequently isolated from dairy products around the world (Peton and Le Loir, 2014).

*Kindirmo* is a full fat or partially skimmed cultured milk that has undergone a heating process, and fermentation by a number of bacterial species from different sources that contaminates the fresh milk.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a main cause of nosocomial infection worldwide. MRSA strains seems to have been picked from clinical care settings and transferred to the community, and have been linked with community-associated infections in humans (EFSA, 2015). Also, in recent times, MRSA has been recognised as an emerging pathogen in livestock, pets, as well as foods of animal origin (Antoci *et al.,* 2013). Humans may acquire livestock-associated MRSA, especially when there is occupational contact with the affected animals (EFSA, 2015). MRSA strains have been recovered from foods of animal origin such as pork, poultry, beef, and milk, indicating that foods serve as reservoir and source of MRSA that spill into the community (Wang et al., 2014). Animals are a source of bacterial zoonosis caused by *Staphylococcus aureus* especially those that seems to lack specific host tropism (Luini *et al.,* 2015).

Food contamination with antibiotics resistant bacteria present great threat to health because the determinants of resistance can be transferred to other bacteria (Jamali *et al.,* 2014). The extensive use of antibiotics in human medicine and livestock management results in the selection and occurrence of antibiotic-resistant bacteria through gene transfer mechanisms, namely, conjugation, transduction, and transformation (Jamali *et al.*, 2015). The frequent use of antibiotics has been linked to the risk of stimulating resistance to antimicrobial agents in bacteria and the transfer of resistant bacteria to man through the food chain (Jamali et al., 2015). Generally, methicillin is not used in routine prophylaxis and chemotherapy in livestock management, but MRSA has been recovered from dairy cattle and dairy products. Moreover, the presence of MRSA in the environment could be one of the sources of MRSA infection in humans since it may survive for several months.

Studying the occurrence and antibiogram of MRSA from dairy product provides invaluable data for preventing and controlling MRSA infection, especially as it relates to foods. In view of the points mentioned above, this study was carried out with the aim of isolating, characterising and determining the antibiogram of MRSA strains from *Kindirmo* in Nasarawa, Nasarawa State, Nigeria.

## MATERIALS AND METHODS

### Description of Study Area

The study was designed to cover some areas around Nasarawa Local Government Area, namely: Nasarawa town, Gunki, Mararraba Udege, and Arabishi (Figure 1).

### Sample Size Determination

The equation below as described by (Naing *et al.,* 2006), was used in calculating the sample size:

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

Where n is the sample size;

'P' is the prevalence from a previous study = 9.0% = 0.09;

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

$$n = \frac{(1.96)^2 \times 0.09(1-0..09)}{0.05^2} = \frac{0.3149}{0.0025} = 126 \text{ samples}$$

It should be noted 9% MRSA prevalence in dairy product from Kaduna metropolis, Nigeria (Usman and Mustapha, 2016) was used in determining the sample size in this study.

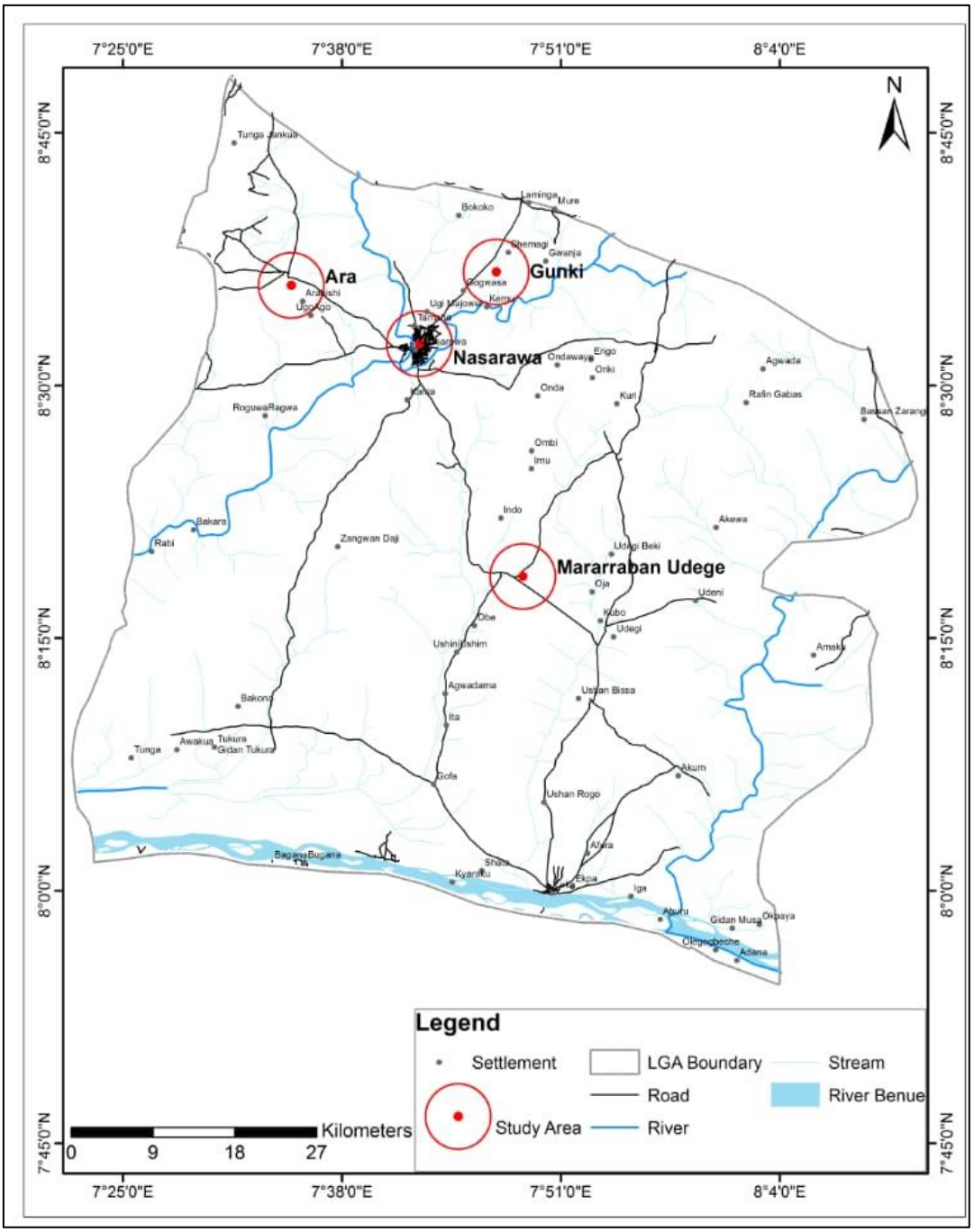


Figure 1: A Map of Nasarawa Local Government Area showing the sampling Areas
Source: Map Gallery, Department of Survey and Geomatics, Federal Polytechnic, Nasarawa

**Collection of Samples**

The sample size was rounded up to 160 in order to ensure even distribution of samples. Forty (40) traditionally-pasteurised milk (*Kindirmo*) samples were randomly purchased from vendors in each of the four areas selected for this study, namely*:* Nasarawa town, Gunki, Mararraban Udege, and Arabishi from January 2021 to April, 2021. The samples were aseptically collected into screw-capped plastic bottles and labelled appropriately. All samples were placed in separate sterile plastic bags to prevent spillage and cross contamination. Samples were then stored in a cooler packed with ice blocks and transported to the Microbiology Laboratory of the Department of Applied Biology/Microbiology, Federal Polytechnic, Nasarawa, Nigeria for analyses.

**Isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA) Strains**

Ten millilitre (10 mL) of each sample was suspended in 90 mL of trypticase soy broth (Oxoid, England) supplemented with 70mg of NaCl/mL and incubated for 18 hours at $37^0$C. Phenotypic isolation of MRSA strains from the samples was carried out by streaking of a loopfuls of enriched samples on to the surface of prepared oxacillin resistance screening agar base (ORSAB) and incubated at $37^o$C for 24 hours. The appearance of bluish colonies on the plates after incubation was indicative of MRSA strains (Usman and Mustapha, 2016).

**Identification of MRSA Strains using Microgen® kits**

The Microgen® Staph-ID kits comprises of a single, microwell test strip, containing 12 standardised biochemical substrates which have been selected on the basis of extensive computer analysis. Each well contained 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.

**Preparation of McFarland turbidity standard**

Zero point five (0.5) MacFarland turbidity standard was used. One (1%) v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water and mixed well. One percent (1%) w/v solution of barium chloride was also prepared by dissolving 1 g of the dehydrated barium salt ($BaCl_2.2H_2O$) in 100 ml of distilled water. Thereafter, 0.6 ml of the barium chloride was added to 99.4 ml of the sulphuric acid solution and mixed well. A small portion of the turbid solution was transferred into a small tube, and was compared with the turbidity of the suspension of the test organism in normal saline (Cheesbrough, 2010).

**Standardisation of inocula**

The concentration of each suspension of the test bacterium and the standard isolate was prepared at 0.5% scale of McFarland's standard (1.5 x $10^8$ cells/mL) in 0.8% normal saline and swabbed over the entire surface of Mueller-Hinton agar (Oxoid) with a sterile cotton swab. A ring of each disc containing single concentrations of each antimicrobial agent was placed on the inoculated surface using sterile forceps. *Staphylococcus aureus* ATCC 25923 which was used as the quality control standard strain. The standard strain was used as the antibiotics susceptible control. The cell suspensions were inoculated by swabbing on prepared Mueller-Hinton agar plates using sterile swab stick. The antibiotic disc was placed on the inoculated medium aseptically with the help of sterile forceps and incubated at $37^0$C for 24 hours. The zones of growth inhibition created by each of the antibiotics against the tested organisms and the standard strain as a positive control was measured to the nearest millimetre using a transparent metre rule. The MRSA strains were classified as 'susceptible', 'intermediate' and resistant according to the interpretative charts of the Clinical and Laboratory Standards Institute (CLSI, 2016).

**Antibiogram of Methicillin-resistant *Staphylococcus aureus* (MRSA) Strains**

Antigiogram of the MRSA strains was evaluated using the agar-disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2016). A panel of eight antibiotics was tested on the MRSA strains. They were: cefoxitin (30 µg), clindamycin (2 µg), chloramphenicol (30 µg), doxycycline (30 µg), gentamicin (10 µg), tobramycin (10 µg), sulphamethoxazole/trimethoprim (25 µg), and vancomycin (Oxoid, England). Categorisation of each bacterium as suceptible, intermediate or resistance to the antibiotics was by comparing the diameter of zone of growth inhibition to the Clinical and Laboratory Standards Institute's (CLSI) breakpoints (CLSI, 2016).

**Multiple antibiotics resistance (MAR) index of the MRSA Strains**

The multiple antibiotics resistance (MAR) index was determined for each of the MRSA strain using the formula: MARI = x/y, where 'x' stands for the number of antibiotics to which the isolate display resistance, and 'y' stands for the total number of antibiotics to which the test organism has been evaluated for sensitivity (Tula *et al.,* 2013).

**Statistical Analyses**

Statistical significant association between the occurrence of MRSA in samples the areas sampled was determined using Chi-square. Probability values of 0.05 were taken as statistically significant for the comparisons.

**RESULTS AND DISCUSSION**

The occurrence of MRSA in *Kindirmo* in the sampling areas are presented on Table 1. No statistically significant association (P = 0.789) was established between the occurrence of MRSA in the samples and the different sampling areas. The occurrence of MRSA as recorded in this study, indicates inadequate or absence of health measures and sanitary practices amongst people involved in milking, milk handling, and distribution. These are the factors that have been adjudged to predispose milk to bacterial contamination. The traditional method of producing dairy products also exposes them to bacteria found on hands, utensils, and clothing of the individuals involved in the production.

The 5.0% of MRSA in *Kindirmo* as recorded in this study was less than the 15 and 7.85% reported by Omoshoba *et al.* (2018) and Umaru *et al.* (2019) in studies conducted to assess the occurrence of MRSA in dairy products in Abeokuta, Ogun State, and Zaria, Kaduna State, Nigeria respectively. Similarly, the percentage occurrence reported in this study was lower compared to the 12.4% reported by Usman and Mustapha (2016) in Kaduna metropolis, Nigeria. Higher percentages of occurrence of MRSA in dairy products at 56.1%, 53%, 8.3% and 7.4%, were reported by Al-Ashmawy *et al.* (2016), Basanisi *et al.* (2017), and Wu *et al.* (2019) in Egypt, in South Italy, and China, respectively. This depicts

MRSA as an important bacterial pathogen in relation to food safety.

Establishing the presence of MRSA in *Kindirmo* in the areas studied is a cause for concern because the dairy product of focus (*Kindirmo*) is commonly consumed in the areas sampled. The findings of this study supports the assertion made by many researchers that dairy products are among the important routes for the transmission of MRSA to humans through the food chain (Jahan *et al.*, 2015).

No statistical association (P = 0.789) was established between the presence of MRSA in *Kindirmo* and the different sampling areas. This shows that the product in the different sampling areas were exposed to the same level of contamination. This may be due to similar or near-similar handling procedures employed in milking, handling, and milk processing. The occurrence of MRSA in processed dairy products implies recontamination during processing or post-processing contamination. Proper treatment and refrigeration which could have minimised the chances of contamination of the product, are completely non-existent in the areas studied. Perhaps, during processing of milk to make *Kindirmo*, the heat applied wasn't high enough to achieve effective pasteurisation. The use of water of doubtful microbiological quality for milking and milk processing has been linked with the risk of contamination of milk with bacteria (Kivaria et al., 2006). Cross contamination of milk and milk products with bacterial pathogens like MRSA can be prevented if milkers and processors observe adequate hygienic practices such as washing of hands and utensils with detergent and clean water.

**Table 1: The Occurrence of MRSA in *Kindirmo* in Nasarawa Local Government Area in Relation to the Areas sampled**

| Sampling Areas | Number Collected | Number Positive (%) | $X^2$ | P-value |
|---|---|---|---|---|
| NWT | 40 | 2(5.0) | | |
| GNK | 40 | 1(2.5) | | |
| MRU | 40 | 2(5.0) | 1.053 | 0.789 |
| ARB | 40 | 3(7.5) | | |
| Total | 160 | 8(5.0) | | |

Keys: NWT = Nasarawa Town, GNK = Gunki, MRU = Mararaban Udege, ARB = Arabishi

The antibiotics susceptibility profiles of the eight (8) MRSA strains obtained is as presented in Table 2. All of the MRSA strains (100%) were susceptible to clindamycin, doxycycline, gentamicin, and vancomycin. Six (6) of the strains demonstrated a relatively high susceptibility of chloramphenicol (75%), five (5) demonstrated susceptibility to chloramphenicol (62.5%), while only three (3) were susceptible to tobramycin (37.5%). The MRSA strains obtained from the samples exhibited 62.5% and 25% resistance to tobramycin and chloramphenicol respectively. The high sensitivity of the MRSA to gentamicin and sulphamethoxazole/trimethoprim as established in this study agrees with the findings of Okpo *et al.* (2018), Ma *et al.* (2018), and Wu *et al.* (2019) in Zaria, Nigeria, and China respectively. The high activity of gentamicin could be linked to its diminished molecular size. This factor enhances the solubility of the antibiotics' solubility in fluids, thereby increasing its penetration power into the cytoplasm where it exerts effects on the ribosome by inhibiting protein synthesis (Poole, 2002). This lends credence to the assertion of (Mailard, 2002) who had argued that, the high efficacy of an antibiotic is linked to its molecular size.

The complete (100%) susceptibility of the MRSA strains to vancomycin in this study is in agreement with the findings of Xu *et al.* (2014) and Wu *et al.* (2019) who conducted different studies on the antibiogram of MRSA from milk and milk products in China. The potency of vancomycin as recorded in this study could be associated with the non-use of the antibiotic in livestock management in the areas studied. The frequent use of an antibiotic in routine therapy and prophylaxis in an area could lead to resistance in bacterial pathogens as a consequence of selective pressure. However, the 100% susceptibility of the MRSA strains to vancomycin as recorded in this study disagrees with the findings of Umaru *et al.* (2016) and Okpo *et al.* (2018), who recorded 61.8% and 71.4% resistance of MRSA from milk and milk products to vancomycin in Zaria. The differences between the findings of this study and the ones mentioned above could be as a consequence of the contamination of dairy products with vancomycin-resistant *S. aureus* obtained from human sources and/or the environment.

This study recorded complete (100%) susceptibility of the MRSA strains to doxycycline. This is in contrast with the reports of Omoshoba *et al.* (2018) who recorded 64.7% resistance of MRSA strains isolated from dairy products to doxycycline in Abeokuta, Nigeria. This may be attributed to the contamination of dairy products in the latter study with doxycycline-resistant MRSA strains derived from human sources or the environment. Another probable reason could be that, doxycycline, which is a derivative of tetracycline, is frequently employed in routine therapy and prophylaxis in livestock management in Abeokuta. And this had resulted in the development of resistance to it due to selective pressure. The high susceptibility (100%) of the MRSA strains to clindamycin agrees with the findings of Ma *et al.* (2018) and Wu *et al.* (2019) who recorded 100% susceptibility of MRSA isolated from dairy products to clindamycin in China. This could be attributed to the non-use of clindamycin in routine prophylaxis and therapy in livestock management which could have promoted the development of resistance due to selective pressure.

The MRSA strains obtained in this study exhibited a relatively high resistance (62.5%) to tobramycin. This is quite surprising because the MRSA strains were completely (100%) susceptible to gentamicin. Tobramycin and gentamicin belong to the same class of antibiotics (aminoglycosides) and exhibit similar mechanisms of activity. This may not be unconnected with the occurrence of tobramycin-resistant MRSA strains have found their way into the product from human sources and/or the environment in the milk product.

The MRSA strains obtained in this study were found to be multi-drug resistant. Multidrug resistance has been defined as resistance of a bacterium to three or more antibiotics in three separate classes (Magiorakos *et al.*, 2012). The multidrug resistance phenomenon recorded in this study is similar to the reports of Umaru *et al.* (2013), Anueyiagu and Isiyaku (2015), Tessema (2016) and Chaalal *et al.* (2016), who recorded different cases of multidrug resistance among *S. aureus* rom milk and milk products in Zaria, Jos, Ethiopia, and Algeria respectively.

Multidrug resistance in bacterial pathogens was partly attributed to the spread of mobile genetic elements which

harbours determinants of antibiotic resistance (Zhao *et al.*, 2001). Determinants of antibiotics resistance in bacterial pathogens can spread within a locality, or between localities as a conquence of selective pressure either in livestock management or humans. Research evidences have shown that antibiotic-resistant bacterial pathogens can be transmitted to humans via the food chain (Jamali *et al.*, 2014).

**Table 2: The Antibiotics Susceptibility Profiles of MRSA from *Kindirmo* in Nasarawa Local Government Area, Nasarawa State, Nigeria**

| Antibiotics | Disc Conc. (µg) | N= 8 | | |
|---|---|---|---|---|
| | | S (%) | I (%) | R (%) |
| Cefoxitin | 30 | 0(0.0) | 0(0.0) | 8(100.0) |
| Clindamycin | 2 | 8(100.0) | 0(0.0) | 0(0.0) |
| Chloramphenicol | 30 | 5(62.5) | 1(12.5) | 2(25.0) |
| Doxycycline | 30 | 8(100.0) | 0(0.0) | 0(0.0) |
| Gentamicin | 10 | 8(100.0) | 0(0.0) | 0(0.0) |
| Sulphamethoxazole/ Trimethoprim | 25 | 6(75.0) | 0(0.0) | 2(25.0) |
| Tobramycin | 30 | 3(37.5) | 0(0.0) | 5(62.5) |
| Vancomycin | - | 8(100.0) | 0(0.0) | 0(0.0) |

Keys: N = number, S = susceptibility, I = intermediate, R = resistance

Table 3 shows the results of the antibiotic resistance patterns of the eight MRSA strains obtained from *Kindirmo* in this study. Six (6) patterns were obtained from bacteria resistant to different combinations of one, two, and three antibiotics. No pattern was found in a combination of four antibiotics and above. Resistance to different combinations of antibiotics by bacteria could be attributed to frequent use and/or abuse of antibiotics in a place which could result in the bacteria becoming resistant to them due to slective pressure.

The multiple antibiotics resistance (MAR) index of the strains is as shown in Figure 2. The five (5) antibiotics resistance patterns of MRSA strains recorded in this study is lower compared to the nine (9), twenty-eight (28), twenty-five (25), and twenty-eight (28) resistance patterns recorded by Usman and Mustapha (2016), Ishaq and Nafi'u (2016), Shiferaw and Ahmad (2016), and Umaru *et al.* (2019). These disparities in the antibiotic resistance patterns reflects the varying levels of use and misuse of antibiotics in the different areas in which the studies were conducted.

**Table 3: The Antibiotic Resistance Patterns of MRSA Strains from *Kindirmo* in Nasarawa Local Government Area**

| Number of Antibiotics | Pattern | Number (%) of Isolates | Area |
|---|---|---|---|
| 1 | CEF | 2(25.0) | NWT, GNK, MRU, ARB |
| 2 | CEF, TOB | 2(25.0) | MRU, ARB |
| 2 | CEF, SXT | 1(12.5) | ARB |
| 3 | CEF, TOB, SXT | 1(12.5) | GNK, MRU |
| 3 | CEF, CHL, TOB | 2(25.0) | MRU |

Key: CEF = Cefoxitin, TOB = Tobramycin, SXT = Sulphamethoxazole/trimethoprim, CHL = Chloramphenicol, NWT = Nasarawa Town, GNK = Gunki, MRU = Mararaban Udege, ARB = Arabishi

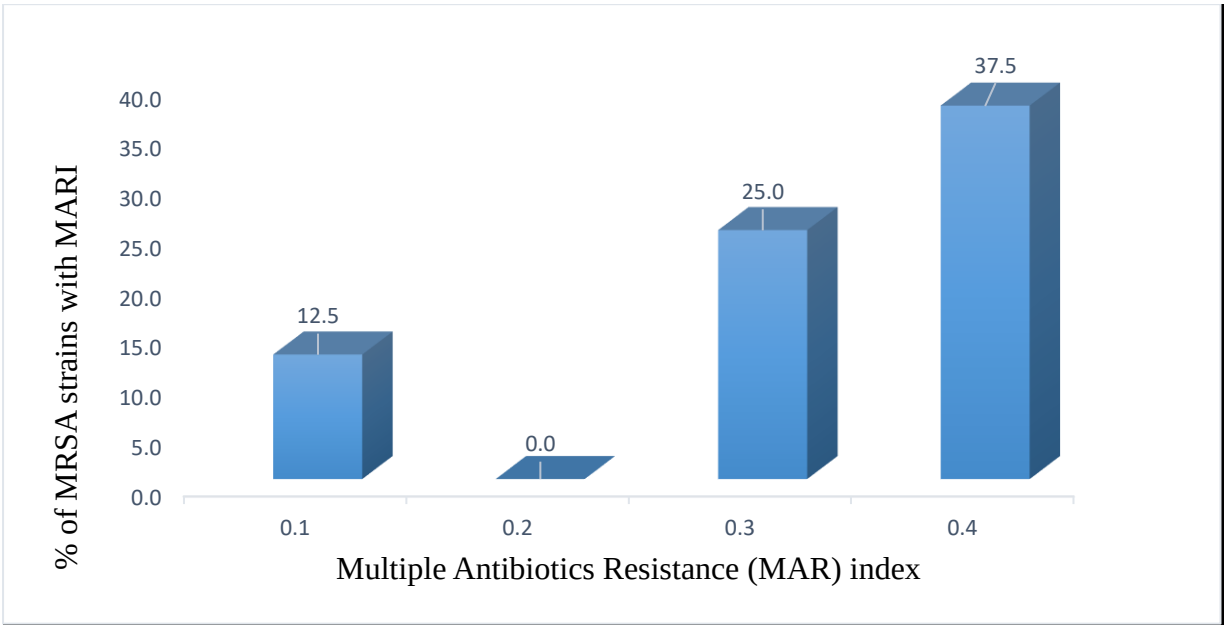


Figure 2: Multiple Antibiotic Resistance (MAR) Index of MRSA obtained from Dairy Product in Nasarawa Local Governmnet Area, Nasarawa State, Nigeria

## CONCLUSION

The detection of multi-drug resistant MRSA strains in dairy product in parts of Nasarawa Local Government Area as recorded in this study indicates that the product constitutes a public health hazard. This is because researches have empirically established the possibility of transmission of antibiotic-resistant bacterial pathogens through the food chain. The antibiogram of the MRSA strains showed the high performance of clindamycin, doxycycline, gentamicin, and vancomycin on the tested isolates. However, the strains demonstrated a relatively high resistance to tobramycin.

## RECOMMENDATIONS

Compliance with basic hygiene requirements in dairy product manufacturing be imposed by relevant authorities. In addition, the concept of hazard analysis and critical control points (HACCP) should be an integral part of an effective hygienic practice at the milk production and distribution points. Routine education of dairy products' sellers on the aspects of milk hygiene practices which will go a long way in enhancing quality standards and safety of the products at the distribution points. This can be accomplished through teaching and training programmes, using participatory approaches. Relevant authorities need to also educate the public on the effects of unnecessary use of antibiotics.

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