

# Isolation and Identification of Methicillin Resistant Staphylococcus Aureus (MRSA) from Traditionally Fermented Milk “Nono” and Yoghurt in Kaduna Metropolis, Nigeria

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## Abstract

This study was to determine the presence of Staphylococcus aureus including the methicillin resistant strains in yoghurt and nono using bacteriological and molecular techniques. Out of 280 samples of yoghurt (140) and nono(140) collected from Kaduna, the overall prevalence of Staphylococcal species were 34(12.14%) . Twenty-nine (85.29%) were methicillin resistant by the Kirby-Bauer disk diffusion method. The 34 staphylococcal isolates were further subjected to Microbact 12S identification system and 9(3.21%) were confirmed as Staphylococcus aureus. All 9(100%) were identified as MRSA using the Kirby-Bauer disk diffusion. The overall prevalence of MRSA was (3.21%). The occurrence of Staphylococcus was higher in nono 24(17.14%) than in Yoghurt 10(7.14%) while prevalence of MRSA was 8 (5.71%) and 1(0.71%) in nono and yoghurt respectively. There was no statistical significant difference between the occurrence of these organisms and the locations ( $P>0.05$ ). All the isolates were resistant to more than one antibiotic but none resistant to all. The isolates showed over 90% resistance to beta lactams such as penicillin, methicillin and oxacillin. The least resistance was to cefixime 13 (38.23%) and 3(33.33%) by both staphylococcal species and MRSA respectively. Resistance by the isolates was by a mec A independent mechanism. Six (66.67%) out of the 9 MRSA were positive for the penicillin – binding protein out of which 5(83.33%) were from nono and 1(16.67%) from yoghurt while 7(76.5%) were positive for betalactamase production out of which 6(85.71%) were from nono and 1(14.29%) from yoghurt. The findings of this study, suggest that nono and yoghurt maybe vehicles for diseases caused by MRSA. Food safety should be advocated by relevant agencies and hygiene among the producers and sellers should be encouraged and enforced.

**Keywords:** MRSA, Nono, Staphylococci, Resistance

## Introduction

Milk accounts for 16% of the total volume of all food products produced from livestock in sub-Saharan Africa (F.A.O., 1986). Fresh milk and its fermented products (nono, yoghurt, kindirmo) constitute good media for microbial multiplication (Jawatz *et al.*, 1991) and hence for transmission of milk borne diseases.

The genus *Staphylococcus* is represented by over 40 species. They are aetiological agents of soft tissue infections and also isolated from anterior nares of healthy individuals. Studies approximate that 30-50% of the human population harbour *Staphylococcus aureus* on their bodies. *S. aureus* produces a variety of extracellular protein, toxins such as exfoliative toxins that are implicated in the staphylococcal scalded skin syndrome (SSSS) in infants (Curran and Al-Salihi, 1980), alpha toxins, beta toxins, delta toxins and bicomponent toxins like Panton valentine leukocidin which is associated with severe necrotizing pneumonia in children and virulence factors which include pyrogenic exotoxins such as staphylococcal enterotoxins (SE) and toxic shock syndrome toxin1 (TSST-1).The SE are proteins which when ingested induce gastroenteritic syndrome, pneumonia, post operative wound infections, nosocomial bacteremia and toxic shock syndrome in humans (Marrack and Kappler, 1990; Horan *et al.*, 1998). *Staphylococcus* can also survive on domesticated animals such as dogs, cats and horses causing suppurative disease, mastitis, arthritis and urinary tract infection and bumble foot in chickens. It can survive for hours on dry environmental surfaces (Whitt and Salyers, 2002).

*Staphylococcus aureus* is known to be notorious in their acquisition of resistance to new drugs and continues to defy attempts at medical control (Talaro and Talaro, 2002). Many strains of *S. aureus* carry a wide variety of multi- drug resistant genes on plasmids (Graham *et al.*, 2005; Ikeagwu *et al.*, 2008). In addition, MRSA are strains considered to be resistant to all beta lactams including ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin- clavulanic acid, piperacillin-tazobactam, cephalosporins, cepheids and other  $\beta$  lactams (such as and carbapenems (Clinical Laboratory Standards Institute, 1997).

MRSA was first reported in UK in 1961 after introduction of methicillin (Voss and Doebbeling, 1995). It is one of the most prevalent nosocomial pathogens and used to be a bacteria found mostly in hospital settings (Hospital associated methicillin resistant *Staphylococcus aureus* known as HA-MRSA) but in recent years, MRSA has attacked healthy people who have contracted it within their communities (Community associated *Staphylococcus aureus* known as CA-MRSA) (www.cdc/eid.org).MRSA infection and colonization have also been reported in horses, dogs, cats, birds, dairy cows and chicken known as Livestock associated methicillin resistant *Staphylococcus aureus*, LA-MRSA. (Lee, 2003; Weese, 2004, 2005). Transmission of MRSA between

animals and humans has been reported (O'Mahony *et al.*, 2005; Fusi *et al.*, 2007; Olonitola *et al.*, 2007; Olowe *et al.*, 2007) as have the recovery of human MRSA from animal contacts (Manian, 2003; Weese, 2004; 2005). *S. aureus* has been detected in milk and other food products locally obtained in Zaria (Umoh *et al.*, 1990a; 1990b; 1990c).

Milk has been reported to be contaminated with MRSA which is suspected to have been acquired from dairy animals or from post milking contamination and poor sanitary practice. Inadequate handling of milk up to the point of pasteurisation allows for the production of thermostable toxins which resist temperature as high as 100°C for 30 minutes (Virgin *et al.*, 2009). Poorly pasteurized milk could be a vehicle for zoonotic disease and there is an inherent danger associated with this product, so the mere touching of raw milk that harbours the bacteria could potentially cause an infection (www. cdc /eid.org, 2008). Even fermentation process with the attendant drop in pH may not rid the product of *Staphylococcus* and this may be transferred to humans (Adesiyun *et al.*, 1995; Headrick *et al.*, 1998). Contamination of milk and its products during processing and distribution can occur and this is a potential threat for the acquisition of *Staphylococcus aureus* including methicillin resistant strains by those who handle foods such as those who buy milk and its products, sellers of the milk and dairy workers.

In northern Nigeria, “nono” is mostly processed by nomadic cattle rearers who are always in close contact with their animals and who usually do not adhere to strict hygiene during processing. Contamination during fermentation of the product can occur during handling and storage (Umoh *et al.*, 1989).

Environmental strains of *Staphylococcus* can re-contaminate milk products even after processing because of addition of poor quality water, from the seller's clothes and hands, exposure to air and use of the same utensils at point of sale (Umoh *et al.*, 1989).

Commercial yoghurt production is more refined and modern methods are utilized during processing but quality control may be compromised due to unsatisfactory storage conditions occasioned by epileptic power supply and the mode of handling during distribution to the final consumers by retailers. Yoghurt deteriorates faster when stored at a higher temperature than at a lower temperature (Hamann and Marth, 1984).

Attempts to control these diseases by chemoprophylaxis and chemotherapy through the use of antimicrobial agents particularly antibiotics which are readily sold over the counter in Nigeria, have resulted in increased prevalence and resistance of *Staphylococcus* to some antibiotics (Nakhla, 1973; Adekeye, 1979; Paul *et al.*, 1982). The widespread indiscriminate use of antibiotics in feed, without prescription among animal and human population has led to the development of resistance by these organisms to most antibiotics and these resistant strains can be shed into the environment and consequently contaminating milk products (Lee, 2003).

## (MATERIALS AND METHODS)

### STUDY AREA

The study was carried out in Kaduna metropolis, Nigeria. Kaduna is one of the largest and most cosmopolitan city in Kaduna state. Kaduna is the capital of Kaduna State in North Central Nigeria with the coordinates 10°31' N 7°26' E 10.517°N 7.433°E (Kaduna state, 2010). Markets in this city are among the largest and enjoy commerce from people within the cities, surrounding cities and from other states. Four markets were randomly selected from among the markets within Kaduna metropolis, (Barnarwa, Station, Central and Kawo) markets. Major markets are those visited on all days of the week with high population of buyers and sellers of nono and yoghurt (i.e where fulanis congregate).

### SAMPLE SIZE DETERMINATION

$$n = \frac{z^2 pq}{d^2}$$

Where n = sample size z = standard normal deviation (1.96), p = anticipated prevalence MRSA = 4.8% by Ghali (2009).

q = 1 - p d = degree of accuracy = 5% = 0.05

$$N = \frac{1.96 \times 1.96 \times 0.048 \times (1 - 0.048)}{(0.05)^2} = 70.2 \text{ (minimum sample size) Thrustfield (1997).}$$

A total of 280 nono and yoghurt samples were obtained for this study.

### SAMPLE COLLECTION/TRANSPORTATION

Nono and yoghurt were collected based on their availability in the market. Adequate quantity of each product was collected in the manner offered to prospective customers and placed aseptically into sterile polythene bag and transported to the laboratory on ice in a coleman box. All samples were processed in the laboratory immediately or kept in the refrigerator at 4°C until processed within 24 hours.

## PROCESSING IN LABORATORY

### A. Enrichment for *Staphylococcus aureus*

Aseptically 10ml of each sample was suspended in 90ml of Trypticase Soy broth (Difco) supplemented with 70mg of NaCl/ ml and incubated at 35<sup>o</sup>c for 20 hours (Lee, 2003).

### B.CULTURE PROCEDURE

Baird Parker agar (BPA OXOID Ltd Basingstoke England) with egg yolk tellurite was prepared according to manufacturer's instruction a day prior use. A sterile wire loop was used to take a loopful of the enriched sample and inoculated onto the agar plate and then streaked out to obtain discrete colonies. The plates were then incubated at 37<sup>o</sup>c for 24 to 48hours. Typical tiny (1-1.5mm), shiny, black colonies were streaked on nutrient agar slants and stored at 4<sup>o</sup>c.

### C. IDENTIFICATION AND CHARACTERIZATION OF SUSPECTED *STAPHYLOCOCCUS AUREUS* ISOLATES

Colonies presumptively identified as *S aureus* were characterized by the underlisted methods. Biochemical identification with Microbact 12S kit test system to confirm *S.aureus* was also carried out and interpreted as recommended by the Manufacturer (Oxoid)

#### i. Gram staining

A smear was prepared on a slide and crystal violet, lugols iodine, alcohol, and safranin were used to flood the slide at recommended times, blot dried and viewed with the microscope. Purple or bluish colonies in clusters were considered to be *Staphylococcus*.

#### ii. Catalase test

A drop of hydrogen peroxide was placed on a slide and a loopful of the suspected isolates emulsified on the slide, release of oxygen seen as bubbles was indicative of a positive reaction.

#### iii. Coagulase test

Test was performed using rabbit plasma by the tube dilution method. Equal volumes of the diluted plasma (1:10) and prepared culture broth were mixed and incubated at 37<sup>o</sup>c. Clot or gel formation was checked at 1, 4 and 6 hourly intervals. Tubes with clot or gel formation were considered to be positive.

#### iv. Urease test

This test checks for the ability of *Staphylococcus* to hydrolyse urea. Urea agar slants were prepared according to manufacturer's instruction and stab inoculated with suspected isolates and incubated at 30<sup>o</sup>c for 24 hours. A change in colour from orange to pink was positive

#### v. Sugar fermentation test

Test was carried out using sugars such as glucose, maltose, mannitol, xylose and lactoselisted above to test for the ability of *Staphylococcus* to ferment some sugars. Each Sugar solution was prepared according to manufacturer's recommendation and Andrade peptone water used as an indicator basal medium. A loopful of a 24 hour incubated broth containing the test organism was inoculated into tubes containing 3mls of prepared Sugar solution and incubated. Tubes with change in colour from pink to yellow were positive indicative of *Staphylococcus*.

#### vi. Haemolysis

20mls of each of rabbit, sheep, human and horse blood were collected into a tube with EDTA and used to prepare blood agar plates as recommended. The organism was obtained from nutrient agar slants and streaked on the prepared blood agar plates using wire loops and then incubated. Alpha haemolysis (incomplete zone of clearance) and beta haemolysis (complete zone of clearance) were observed in rabbit and sheep blood while no haemolysis (delta and gamma) were observed in horse and human blood. Organisms showing this type of haemolysis were considered *Staphylococcus*.

### D. SUSCEPTIBLY TESTING OF ISOLATES TO VARIOUS ANTIMICROBIALS.

Phenotypic methicillin or oxacillin resistance of *S. aureus* was determined by an agar disc diffusion method according to recommendations of the Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton Agar (Lee, 2003). Antibiotics included in the panel were methicillin 10µg, oxacillin 1µg, tetracycline 10µg, vancomycin 30µg, ampicillin 10µg, cefixime 5µg, amikacin 30µg, chloramphenicol 30µg, ciprofloxacin 5µg, erythromycin 15µg, gentamicin 10µg, penicillin 10µg and trimethoprim-sulphamethoxazole 25µg. Mueller Hinton agar was prepared according to manufacturer's instruction and broth culture that had been adjusted to mac farland (0.5%) was used to flood the prepared plate. An antibiotic dispenser was used to dispense the antibiotics onto the plate and incubated for 18hours. Zones of inhibition were measured to the nearest millimetre and compared to the CLSI guide and further recorded as resistant or susceptible.

Also presumptive determination of methicillin resistance was carried out using commercial media, ORSAB agar (OXOID). ORSAB was prepared according to manufacturer's instruction. The ORSAB

supplement was also included in the preparation. The test isolate was streaked and incubated at 37°C for 24 hours. Bluish color was positive for presumptive methicillin resistance.

## 2.5 MOLECULAR CHARACTERIZATION

Presence of *mec A* gene was verified for the MRSA isolates by means of PCR using primers:

*mec A1* 5'- AAA ATC GAT GGT AAA GGT TGG C- 3' (forward primer)

*mec A2* 5'-AGT TCT GCA GTA CCG GAT TTG C - 3' (reverse primer) (Lee, 2003)

### A. MATERIALS FOR POLYMERASE CHAIN REACTION

DNA extraction was carried out according to steps in the instruction manual of the ZYMO research kit (ZR, Pretoria, S.A. Inqaba Biotec). PCR was carried out in a 50 µl reaction consisting of 25 µl Mastermix, 4 µl Primers, 14 µl Water and 7 µl DNA. Amplification was carried out using 40 cycles of amplification at 94°C for 30 secs, 55°C -70°C for 30 secs and 72°C for 1 minute, followed by 5 minutes of an additional extension at 72°C and an initial denaturation of 95°C for 5 minutes. PCR products were electrophoresed on a 1.5% agarose gel and stained with ethidium bromide to view DNA band of 533 bp which represents a fragment of *mecA* gene (Lee, 2003).

## PROTOCOL FOR DETERMINATION OF PENICILLIN- BINDING PROTEIN (PBP2A) AMONG MRSA ISOLATES

Overnight culture of isolates on Mannitol salt agar was used for the test. Four drops of extraction reagent 1 was put into the micro centrifuge and a sterile loop to pick the grown isolates and emulsified in the tube. The tubes were then heated at 95°C in a water bath for 3 minutes. This was allowed to cool to room temperature and a drop of extraction agent 2 added. This was then centrifuged at 1500 rev for 5 mins. A drop of the supernatant was added to the round circles on the test papers and a drop of latex reagent added the test paper then rotated gradually to check for agglutination. Presence of clumps or granules against a cloudy background was recorded as positive while a homogenous suspension was recorded as negative.

## PROTOCOL FOR THE DETERMINATION OF BETA LACTAMASE ENZYMES AMONG MRSA ISOLATES

The can holding the beta lactamase impregnated sticks were removed from the freezer and allowed to reach room temperature. The colored end of the beta lactamase stick was used to pick a mass of well isolated colony. This test requires moisture and one or two drops of distilled water was used to moisten the coloured end of the stick. The sticks were then examined for 5 minutes, then 15 minutes and an hour for colour change. A positive reaction is shown by the development of a pink-red while no colour change is observed with organisms that do not produce lactamase.

## DATA ANALYSIS

Data collated at the end of the study were analysed using Statistical package for Social Sciences (SPSS) version 17.0 (SPSS Inc. Chicago, IL, USA). Statistical methods employed included descriptive statistics using percentages, tables and graphs. Chi-square of Independence was used to establish association between variables. Values of P less than 0.05 were considered significant.

## RESULTS AND DISCUSSION

A total of 34 isolates of *Staphylococcus* spp were identified based on conventional biochemical tests giving a prevalence rate of 12.14%. Microbact<sup>®</sup> 12 S identification kit system revealed a total of 9 *S.aureus* species yielding a prevalence of 3.21 % (Table 1). This is low compared to the 37-43% and 81.3% reported by Umoh (1989) and Umoh *et al.* (1990b) respectively. It is also low compared to 12.6%, 15.9 and 14.7% reported by Ghali (2009), Zouharova and Rysanet (2009) and Strastpova *et al* (2000) in Nigeria, Turkey and Czech Republic respectively. Although, cognizance should be taken of the other *Staphylococcus* species that were isolated which could have contributed to the low prevalence of *S.aureus* in this study. This difference may be due to the fact that fermentation of these products could have created an acidic condition that denatures bacteria hence affecting their growth. Samples were collected in this study during the dry period of December to April in which prevalence of certain organisms was low and Ph of milk tended to be low (Umoh *et al.*, 1990b). The prevalence of MRSA (3.21%) in this study is in agreement with the 4.8% obtained by Ghali (2009), but lower than 29.3% obtained in China by Lee (2003). This could be due to larger sample size and the type of samples in the various studies.

There was no statistically significant difference ( $P > 0.05$ ) between location and occurrence of *S. aureus* and MRSA, although the strength of association between MRSA and these locations were high ( $OR > 1$ ), it was not significant (Table 1).

In Kaduna, Station market had the highest prevalence of 18.6%, 5.71%, 5.71% respectively for



*Staphylococcus* species, *S.aureus* and MRSA (Table 1). The total prevalence of *Staphylococcus* in yoghurt and nono in all the markets in Kaduna were 12.14% (Table 1). There was a statistically significant association between the occurrence of *Staphylococcus* and markets in Kaduna ( $P < 0.05$ ) which was marginal and this may have been due to the slight increase in number of the organism obtained from central and station markets. Incidentally these markets are more populated and always open. In Kaduna, the prevalence of *Staphylococcus* in nono was 17.14% while in yoghurt it was 7.14%; while for *S.aureus* and MRSA, prevalences were 5.71% and 0.71% in nono and yoghurt respectively. There was no statistically significant association between the occurrence of these organisms and location indicating that location had no role in occurrence of the organism but contamination of the products in the markets maybe a major contributory factor (Tables 2&3).

**Table 1 Prevalence of *Staphylococcus* and MRSA in Kaduna**

Location	size	Staph(%)	<i>S.aureus</i> (%)	MRSA(%)	OR	CI at 95%
Barnawa	70	7(10)	1 (1.43%)	1(1.43%)		
Central	70	11(15.7)	3(4.28%)	3(4.28%)	3.09	0.3133-30.47
Station	70	13(18.6)	4 (5.71%)	4(5.71%)	4.18	0.4552-38.41
Kawo	70	3 (4.3)	1(1.43%)	1(1.43%)	1.00	0.0613-16.32
Total	280	34(12.14)	9(3.21%)	9(3.21%)		

Staph  $\chi^2 = 7.901$   $p = 0.0481$   $P < 0.05$

MRSA  $\chi^2 = 3.10$   $p = 0.3765$   $P > 0.05$

Key: No – Number sampled, Staph+ve – *Staphylococcus* positive, *S.aureus*+ve – *Staphylococcus aureus* positive, MRSA+ve- methicillin resistant *Staphylococcus aureus* positive,  $\chi^2$  - chi square

**Table 2: Prevalence of *Staphylococcus* and MRSA in Nono in Kaduna**

Location	No	Staph+ve(%)	<i>S.aureus</i> +ve(%)	MRSA+ve(%)	OR	C.I
Barnawa	35	4(11.4)	1(2.86)	1(2.86)		
Central	35	10(28.57)	3(8.57)	3(8.57)	3.188	0.3149-32.26
Station	35	7(20)	3(8.57)	3(8.57)	3.188	0.3149-32.26
Kawo	35	3(8.6)	1(2.86)	1(2.86)	1.00	0.06-16.66
Total	140	24(17.14)	8(5.71)	8(5.71)		

Staph  $x^2 = 6.034$   $p = 0.1099$

MRSA  $x^2 = 2.121$   $p = 0.5476$

**Table 3: Prevalence of *Staphylococcus* and MRSA in yoghurt in Kaduna**

Location	No	staph (%)	<i>S.aureus</i> (%)	MRSA (%)
Barnawa	35	3(8.6)	0(0)	0(0)
Central	35	1(2.9)	0(0)	0(0)
Station	35	6(17.14)	1(2.85)	1(2.85)
Kawo	35	0(0)	0(0)	0(0)
Total	140	10(7.14)	1(0.71)	1(0.71)

Nono from central and station markets had the highest contamination rate of *S.aureus* and MRSA (8.71%) as compared to yoghurt from central market (2.9%). Yoghurt from station market had a surprisingly higher occurrence of *S.aureus* and MRSA (17.14%) (Table 2 & 3). This maybe due to continuous thawing and freezing of a particular batch of yoghurt that may have been contaminated either during production or distribution.

Nono had the highest isolation rate of *Staphylococcus* species 24(17.14%) and that of yoghurt was 10(7.14%) The isolation rate of *S.aureus* and MRSA were higher in nono 8(5.71%) than in yoghurt 1(0.71%) (Table 4) .There was no significant difference in the occurrence of *S.aureus* and MRSA in nono and yoghurt ( $P > 0.05$ ), although there was a high strength of association between MRSA and its occurrence in nono and yoghurt ( $OR > 1$ ) and was significant (Table 4). The lower prevalence of these organisms in yoghurt compared to “nono” could be due to better hygienic practices during production, distribution, packaging, storage and the need by the manufacturers to protect a brand.

Other species of *Staphylococcus* isolated were as follows: *S.intermedius* (17.64%), *S.hyicus* (44.12%) *S.epidermidis* (2.94%), *S.chromogenes* (8.82%).

**Table 4: Prevalence of Staph and MRSA based on sample type**

Sample type	No	staph(+ve)	<i>S.aureus</i> (+ve)	MRSA
Nono	140	24(17.14)	8(5.71)	8(5.71)
Yoghurt	140	10(7.14)	1(0.71)	1(0.71)
Total	280	34(12.14)	9(3.21)	9(3.21)

Staph  $\chi = 6.561$   $P = 0.0104$   $OR = 2.690$   $95\% CI = 1.234-5.863$

MRSA  $\chi = 5.625$   $P = 0.0847$   $OR = 8.424$   $95\% CI = 1.039- 68$

### Susceptibility of Isolates to Antimicrobial Agents

All the 34 *Staphylococcal* isolates were tested for susceptibility to 13 antimicrobial agents and the isolates showed the following percentage resistances as shown in (Figure 1). All 9 MRSA were resistant to more than one of the 13 antimicrobials tested including the beta lactam drugs. A hundred percent resistance to methicillin, penicillin and oxacillin were recorded. Relatively low levels of resistance were observed for cefixime and tetracycline meaning this could be possible drugs for choice for these strains of MRSA (Figure 2). The susceptibility of some of the isolates to sulphamethoxazole/trimethoprim maybe an indication of community associated MRSA. Mixed fermentation has been reported to occur in nono as fermentation is not controlled and different organisms are present at a time, as a result, transfer of resistance between organisms can occur and this may have accounted for the high resistance seen in nono as compared to yoghurt where only one known bacteria culture is used during fermentation. The percentage resistance to vancomycin is alarming and has been widely reported internationally (Fridkin, 2001; Hiramatsu *et al.*, 1997) even in Nigeria (Olayinka *et al.*, 2005). Culturing on ORSAB medium revealed that 22(64.7%) of the isolates were presumptively identified to be methicillin resistant.

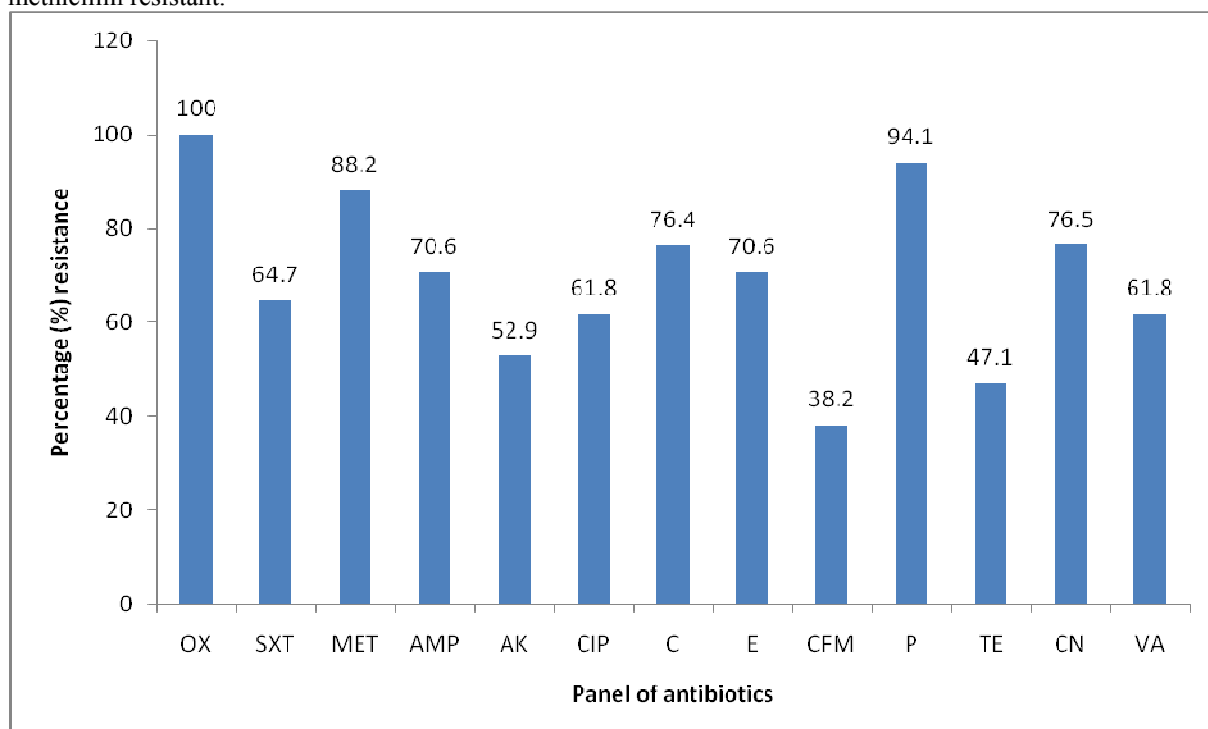
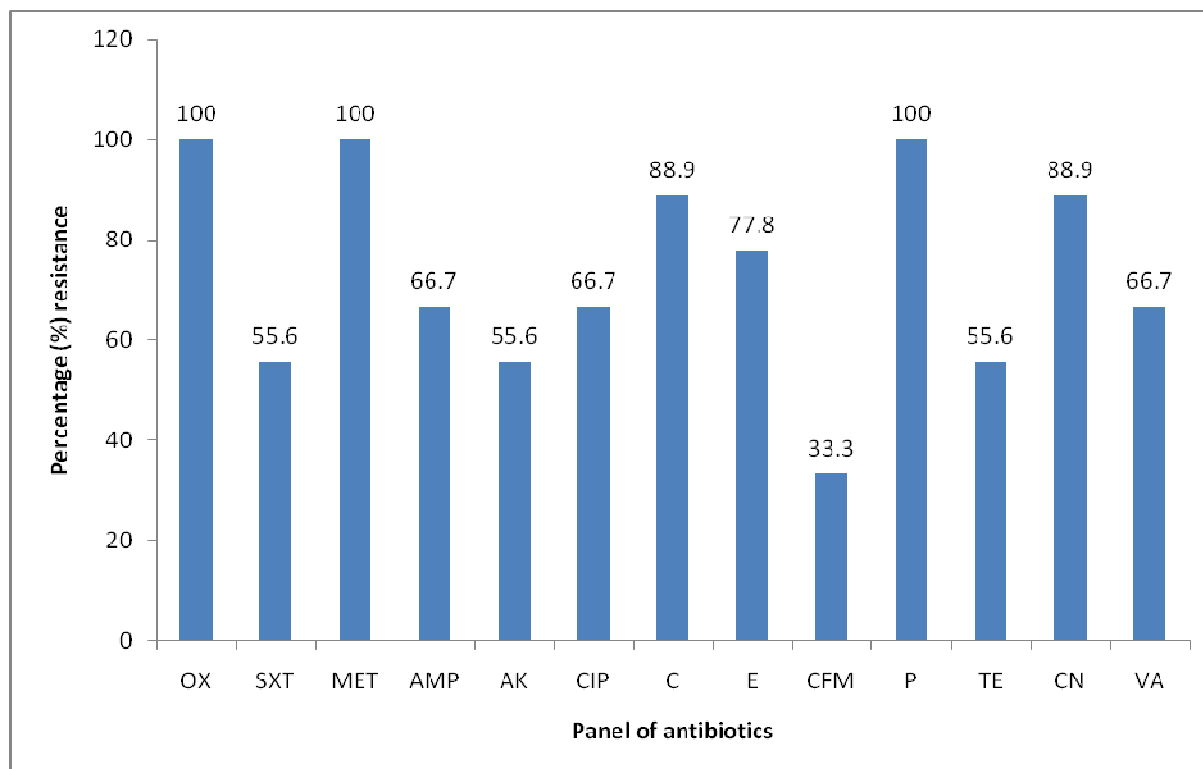


Figure 1: Percentage Resistance of *Staphylococci* spp to antimicrobial agents (n=34)



**Figure 2 Percentage Resistance of *Staphylococcus aureus* (MRSA) to antimicrobial agents (n=9).**  
 All 9 MRSA isolates showed multiple antimicrobial resistance patterns (Table 5).

**Table 5: Multiple Drug Resistance Profiles and MAR Index of 17 MRSA Isolates**

Isolates No	Antimicrobial profile	MAR Index
KDNB6	OX, SXT, MET, CIP, C, E, P, CN, VA	0.69
KDNCI	OX,SXT,MET,AMP,AK,CIP,C,CFM,P,TE,CN	0.85
KDNC24	OX, SXT, MET, AMP, C, E, P, TE	0.62
KDNC30	OX,SXT,MET,AMP,AK,CIP,C,E,P,TE,CN,VA	0.92
KDNS1	OX, MET, AMP, AK, CFM, P, TE, CN, VA	0.69
KDNS4	OX,MET,AMP,AK,CIP,C,E,CFM,P,CN,VA	0.85
KDNS5	OX, MET, CIP, C, E, P, CN	0.54
KDYS20	OX, MET, C, E, P, CN, VA	0.54
KDNK9	OX,SXT,MET,AMP,AK,CIP,C,E,P,TE,CN,VA	0.92

**Table 6: Haemolytic Patterns of *Staphylococcus aureus* and MRSA isolates**

RBC	No (%) of isolates with different Haemolytic patterns*			
	$\alpha$	$\beta$	$\gamma$	$\delta$
Horse	0(0)	4(44.4)	0(0)	5(55.6)
Human	1(11.1)	2(22.2)	6(66.7)	0(0)
Rabbit	5(55.6)	4(44.4)	0(0)	0(0)
Sheep	4(44.4)	5(55.6)	0(0)	0(0)
Total	10	15	6	5

Numbers in parathesis are in percentages \* $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  haemolysis

#### Detection OF *mec A* gene by Polymerase Chain Reaction (PCR)

None of the 9 *S. aureus* was found to be positive for *mec* by PCR. The absence of *mec A* gene among isolates tested in this study suggests that other factors may be responsible for resistance. It may also be necessary to combine detection of *mecA* gene with detection of Pbp2a, homologues of *mec A* such as *mecC*, mobile elements, transposons and phages which can harbour other genes responsible for resistance. Primers used in this study were specific for the amplification of *mecA* gene.

#### Detection of Pbp2a and Beta-Lactamase

Six (66.67%) out of the 9 MRSA were positive for the penicillin – binding protein out of which 5(83.33%) were from nono and 1(16.67%) from yoghurt while 7(76.5%) were positive for betalactamase production out of which 6(85.71%) were from nono and 1(14.29%) from yoghurt.Pbp2a is a product of *mec* element and its detection among the isolates portending that a likelihood of a homologue of the *mec A* gene.

## CONCLUSION

A cross-sectional study was carried out to determine the occurrence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in yoghurt and “nono” in Kaduna. Overall prevalence of *Staphylococcus* species was 34 (12.14%) using conventional biochemical tests, while 9(3.21%) were confirmed to be *Staphylococcus aureus* and MRSA by the Microbact 12S identification system and antibiotic susceptibility testing respectively. Twenty two (64.7%) out of the 34 staphylococci isolates were positive for methicillin resistance when tested with Oxacillin resistant *Staphylococcus aureus* basal medium(ORSAB) while 7 ( ) out of the 9 *Staphylococcus aureus* isolates were positive on ORSAB. All the 34 staphylococcal isolates showed high level of resistant to more than one of the antibiotics tested including the beta lactams such as methicillin ,oxacillin and penicillin with the least resistance to cefixime (38.2%).All the 9 *Staphylococcus aureus* and MRSA isolates were resistant to methicillin, oxacillin and penicillin (100%) while the least resistance by the isolates was to cefixime(33.3%). Cefixime is the drug of choice considering that fewer isolates showed resistant to them. None of the 9 MRSA harboured the methicillin resistance gene *mec A* gene by PCR, but 6 (66.67%) and 7(76.5%) were positive for Pbp2a and betalactamase respectively suggesting that resistance by strains of *S.aureus* in this study was by Pbp2a and beta-lactamase production. Consequently, findings from this study suggest that nono and yoghurt can serve as vehicles of transmission of food borne diseases and are a food safety problem. The study also suggests that *Staphylococcus aureus* and MRSA is becoming increasingly resistant to drugs commonly used in humans and animal population and even to drugs previously thought to be effective against the organism.

## RECOMMENDATIONS

From the results of this study, the following recommendations were made:

1. Nono and yoghurt producers should be encouraged and educated on how to undertake proper fermentation and pasteurization of their products to decrease the presence of *Staphylococcus aureus* and MRSA in the products. This can be achieved through workshops, seminars, fulani day, herd and farm visits by the relevant authorities.
2. Hygienic and healthy sanitary practices such as washing of utensils, hands, covering of food and cleanliness on the part of the seller should be maintained through the chain of production until it gets to the final consumer.
3. There is need to include different groups of antibiotics in the panel during susceptibility testing of MRSA to antimicrobials in order to arrive at the best choices of treatment of MRSA infections
4. New diagnostic guidelines for the detection of MRSA should consider tests for homologues of the *mecA* gene and other factors (such as production of resistance proteins and enzymes) in strains isolated from Nigeria.

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