



Isolation and Characterization of MRSA from Locally Processed Meat Hawked in Gombe, Nigeria

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Abstract

Three types of locally processed meat products were collected from vendors across hawking points in Gombe town. Stick meat (*tsire*) 30 samples, Roasted meat (*balangu*) 30 samples and dried meat (*kilishi*) 15 samples making 75 samples. Samples were taken to the Microbiology Laboratory of Gombe State University and analyzed for the presence of methicillin resistant *Staphylococcus aureus* (MRSA) through culturing, biochemical tests and DNA analysis. The isolated *Staphylococcus aureus* were subjected to antimicrobial susceptibility test using standard antibiotics. The result showed 13.33% of the total isolates to be Methicillin Resistant *Staphylococcus aureus* as confirmed by the presence of *mecA1*, *mecA 2* and *pvl* genes by polymerase chain reaction. This is of great health importance to the public.

Key words: Meat, Methicillin, resistance Antimicrobial Cefoxitin, MecA1

INTRODUCTION

Staphylococcus is a human pathogen, causing infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumonia, osteomyelitis, endocarditis as well as toxin mediated syndromes such as toxic shock syndrome (TSS) and food poisoning (Shittu *et al.*, 2011). *Staphylococcus aureus* is able to cause a large diversity of both benign and lethal infections in humans and animals because of a wide range of virulence factors that include various toxins and enzymes (Bal and Gould, 2005). It has emerged as one of the most important human pathogens and has become a leading cause of hospital and community acquired infections (Shittu and Lin, 2006).

Studies on the microbiological quality of some meat products such as stick meat (*tsire*) and dried meat (*kilishi*) have shown them to have organisms of public health concern (Igene, *et al.*, 1989).

Staph. aureus is one of the more common causes of food poisoning. Commonly affected foods include processed meat,

custard pastries, potato salad and ice cream that have been contaminated with the bacteria from human skin (Robert, 2004).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a leading cause of hospital-acquired infections worldwide accounting for more than 60% of *S. aureus* isolates in hospitals in the United states (Ayepola, 2012.) Cases of MRSA infections have been documented among healthy community-dwelling persons without the established risk factors for MRSA infections. This work is aimed at determining the occurrences of MRSA in Gombe and environs

Materials and methods

Sample collection

A total of 75 samples of three different types of roasted meat snacks were collected from meat vendors by direct buying. The samples were collected in sterile polythene bags, double wrapped in another polythene bags to avoid air contamination and brought immediately to the microbiological laboratory of Gombe state University for MRSA screening.

Isolation of *S. aureus*

In the laboratory, 25g of meat sample was homogenized in 225ml peptone water supplemented with 7% Sodium Chloride (NaCl) were prepared. The samples were aseptically blended with a blender, after which the blended samples were poured into a labeled conical flask and incubated at 37°C for 24hrs.

Isolation on Mannitol salt agar (MSA) (TM Titan Biotech Ltd): This was used to screen for *S. aureus* due to the organism's ability to ferment mannitol and to grow on agar containing 70–100 g/l sodium chloride, this is a selective criterion of MSA, to observe mannitol utilisation is confirmed by the resulting medium change to yellow from its initial pink colour (Shamsuddeen 2016).

Isolation on Baird Parker agar (BPA) (Biomark Laboratories. Ltd).

Baird Parker Agar Base was supplemented with 50ml egg yolk and Potassium Tellurite for each 63g which was dissolved in 950ml distilled water. From the MSA, a colony was picked using a wire loop, which was introduced on the BPA and incubated at 37°C for 24hrs. On BPA all colonies that appeared black were later picked and inoculated on Blood agar(TM Titan Biotech Ltd) for Haemolysis observation (FAO, 1979)

Isolation on Blood agar (TM Titan Biotech Ltd):

Colonies from the BPA were inoculated by streaking on to Blood Agar (BA) using a wire loop and the plates were incubated at 37°C for 24 hrs. The *S. aureus* produced yellow to cream 1–2 mm in diameter colonies after overnight 24hrs incubation which were *beta* haemolytic (Cheesebrough, 2008).

Isolation on Cysteine electrolyte deficient (CLED) agar(TM Titan Biotech Ltd):

From the MSA plate colonies were picked and were re-introduced on CLED using a wireloop and the plates were incubated at 37°C for 24hrs. Deep yellow colonies were produced with a total change of the medium colour from green to complete yellow (Cheesebrough, 2008).

Antimicrobial susceptibility testing

Susceptibility of the isolates to cefoxitin was tested using disc diffusion method as standardized by CLSI 2014 (Clinical and Laboratory Standards Institute, 2014).

Molecular analyses

DNA extraction (using Instagene Matrix, Biorad®)

The DNA extraction and preparation for Polymerase Chain Reaction and electrophoresis was carried out at Veterinary teaching Hospital of Ahmadu Bello university, Zaria. The black colonies on the BPA supplemented with cefoxitin were inoculated on Lysogeny broth (LB) medium prepared in the laboratory which contain yeast extract 0.5g, NaCl 0.5g, Peptone Water 0.5g, NaOH, which was dissolved in 100ml distilled water. The samples were inoculated into the medium and incubated at 37°C for 24hrs. 1.0ml of the organisms suspension were pipetted each into appendorf tubes, centrifuged at 10000rpm/2min and the supernatant was discarded., 500ul of lyses buffer was added and the tubes were incubated at -37°C for 30min in J R Selecta 5a incubator. The appendorf tubes were later vortexed and centrifuged at 10000rpm for 2mins. Later 400ul of protease solution was later added and incubated as 37°C/30min.

The content of the appendorf tubes were transferred into spin column and centrifuged at 10000rpm/10min and 400ul pre-wash buffer solution was added and centrifuged again at 10000rpm/1min and 200ul wash buffer was added and centrifuged, later 10000rpm, 100ul DNase elution buffer was added to elude the DNA down into the spin tube that were inserted into the appendorf tubes and centrifuged until a clear DNA solution was realised which was preserved at -20°C.

Gel electrophoresis

From the DNA extract, 15ul was mixed with a 6x DNA Loading dye for conventional colour tracking in DNA migration. Agarose gel was poured into chamber of PowerPC HC Biorad electrophoresis machine.

Comb was removed from the solidified gel and the gel was inserted into the chamber then later 5x buffer was poured. The DNA extract mixture was pipetted into the comb holes and later the machine was set at 75°C for 40min. This was done to make sure that the DNA extract surely contains DNA.

Primers used

1. Staph756F (5- AACTCTGTTATTAGG GAAGAACA-3)
2. mecA1 (5' - GTA GAA ATG ACT GAA CGT CCG ATA A - 3')
3. mecA2 (5' - CCA ATT CCA CAT TGT TTC GGT CTA A - 3')
4. spa (5' - CGC TGC ACC TAA CGC TAA TG - 3')
5. pvl-F (5' - GCTGGACAAAATTCTTGGAAAT AT - 3')

These Primers were used for the amplification of the fragments of the methicillin-resistant gene (mecA).

Positive control used was *S. Aureus* (Staph756F). Also, negative control was used. By adding DNA of a fungi (Wichelhaus *et al.*, 2001).

Polymerase Chain Reaction (PCR)

Master Mix 12.5ul x number of samples
 Primer 1ul/sample
 Water 0.5ul/sample
 Template 7.0ul/sample

All the PCR tubes were centrifuged to mix and the tubes were later loaded into the PCR machine and set to 40 circles.

Each circle has five steps with its respective time. The first was the initial denaturing temperature of the DNA denoted with 95.0 which lasted 5min, the second was the denaturing temperature denoted as 94.0 which lasted for 30 seconds, the third denoted 55.0 which lasted for 30 second was the annealing temperature where the targeted gene and the primers meet, the forth was the extension temperature denoted 72.0 which lasted for 30 seconds where in the extension along the sequence occurs and finally the final extension denoted as 4.0 which marks the synthesis of the complement gene sequence can last to infinity after the final circle

After the 40th circle the contents of the PCR tubes were subjected to electrophoresis again for 45min, removed and put into gel documentation machine Biorad universal hood which was connected to a computer system, using a computer programme the result was printed on the computer screen.

RESULTS:

Of the 30 suya, 30 balangu and 15 kilishi samples tested, 9, 17 and 15 yielded *Staphylococcus aureus* respectively as presented in Table 1., with their respective characteristics on the different media used.

Table1: Isolation of *Staphylococcus aureus* on different media from the different meat products

S/T	N	Growth	MSA	BPA	CLED	BA	CaT	CoT	Inference
Tsire	30	09(30%)	Yellow	Black	Yellow	Haemolysis	+	+	<i>S. aureus</i>
Balangu	30	17(56.7%)	Yellow	Black	Yellow	Haemolysis	+	+	<i>S. aureus</i>
Kilishi	15	15(100)	Yellow	Black	Yellow	Haemolysis	+	+	<i>S. aureus</i>
Total	75	100(100%)							

Key

- S/T: Sample type
- N: number of samples
- MSA: Mannitol salt agar
- BPA: Baird Parker Agar
- CLED: Cysteine Electrolyte Deficient agar
- BA: Blood agar
- CaT: Catalase test
- CoT: Coagulase test

Out of the *Staphylococcus aureus* isolates from the meat products, 2, 3, and 5 isolates from suya, Balangu and kilishi respectively were confirmed to be MRSA based on resistance to cefoxitin.

Table 3: Antimicrobial susceptibility of the isolates to cefoxitin single disc

Source	Number of samples	Susceptibility			MRSA
		S	I	R	
Tsire	09	4	3	2	2(22.3%)
Balangu	17	8	6	3	3(17.6%)
Kilishi	15	6	4	5	5(33,34%)
Total	41				10(13.33%)

Key

S: Sensitive; zone diameter of ≥ 28 mm
 I: Intermediate; zone diameter of 24–27mm
 R: Resistant; zone diameter of ≤ 23 mm
 MRSA: Methicillin Resistant *Staph. aureus*

DNA analysis for detection of mecA gene

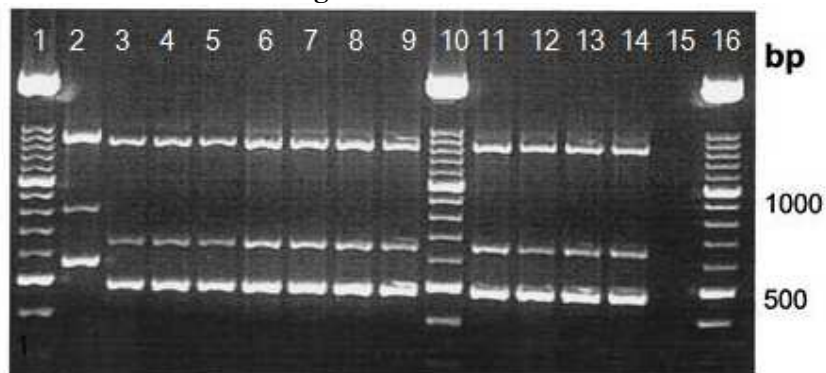


Plate 1. Bands of mecA gene after separation on Agarous Gel Electrophoresis

1, 10 and 16 were the ladder, 2 positive control. Band 1 was the mecA1, the second mecA2 and the third was the PVL, column 2 to 14 were samples while 15 was negative control.

Discussion

The results of this study have confirmed the occurrence of MRSA in *tsire*, *balangu* and *kilishi* with the highest occurrence in *Kilishi*. This may be due to the fact that antimicrobial resistance come due to exposure to conventional drug (WHO 2015) and nowadays cattle and sheep are always exposed to antimicrobial during vaccines and when infected with a disease.

The presence of MRSA on the meat products may be as a result of human contamination of the meat either during processing or in the spices used or at the point of sale in the market. This idea is in line with what was inferred by Obrien *et al.*,

2012) who showed in their studies that even the USA markets that are advanced could not protect their finished meat product from after processing contamination with MRSA especially pork products. Contamination by handlers may occur especially in the tropical regions of the world more frequently due to the rise in temperature where a seller can use the back of the hand or handkerchief to relieve his sweat and use the same hand to cut meat especially the kilishi where hands are used in cutting instead of knives. Weese *et al* (2010) has shown that, the contamination of food (meat) with sweat is possible.

The idea of passing resistant gene across species was earlier discussed by Olatu *et al.*, (2015) where it was reported that due to surface contamination by the MRSA there may be a possibility of the organism passing the resistance gene across species.

The human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora and in the nostrils. *S. aureus* is the most common species of *Staphylococcus* to cause Staphylococcal infections and is a successful pathogen due to a combination of nasal carriage and bacterial immunoevasive strategies (Kluytmans *et al.*, 1997). *Staphylococcus aureus* (*S. aureus*) has long been recognized as one of the most important bacteria that cause disease in humans. (Kluytmans *et al.*, 1997) It is the leading cause of skin and soft

tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most Staphylococcal infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections (bacteraemia), pneumonia, or bone and joint infections. (Boucher *et al.*, 2010).

Conclusion

Conclusively this study has succeeded in isolating *S. aureus* from Suya, Balangu and Kilishi within selected areas of Gombe town in Gombe State. Also from the sample collected MRSA was isolated in all the three sources.

Recommendation

All meat handlers and the general public should exercise strict personal and environmental hygiene in all operations to avoid cross contamination of food products

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