Food & Nutrition Journal
Daworiye PS, et al. Food Nutr J. 6: 246.
https://www.doi.org/ 10.29011/2575-7091.100146
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Research Article

The Incidence of *Staphylococcus aureus* in Fresh Meat sold at Evening Markets within Yenagoa Metropolis, Bayelsa State, Nigeria

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Citation: Daworiye PS, Soroh EA, Enaregha EB, Tonbofah R (2022) The Incidence of *Staphylococcus aureus* in Fresh Meat sold at Evening Markets within Yenagoa Metropolis, Bayelsa State, Nigeria. Food Nutr J 7: 246. DOI: 10.29011/2575-7091.100146

Received Date: 03 May, 2022; **Accepted Date:** 23 May, 2022; **Published Date:** 26 May, 2022

Abstract

Meat provides a major source of protein for humans. Preparation and handling of meat by sellers, buyers and consumers can expose meat and man to infection by *Staphylococcus aureus*, an opportunistic pathogen. Samples obtained from market sellers were prepared and cultured on nutrient agar and mannitol salt agar and subjected to biochemical tests and molecular identification. Total bacterial count was within the range of 4.0 x 10^7 to 1.03 x 10^9 CFU/g. The differences in bacterial growth observed on nutrient agar and mannitol salt agar was significant at p(0.05), r= 0.529. The biochemical tests of isolated organisms showed that two out of the five isolated organism were Gram positive, Coagulase positive, Catalase positive, Methyl red positive and Citrate positive *Staphylococcus aureus*. Molecular identification using PCR assay showed a match with *Staphylococcus aureus* strain UA899. The presence of this bacteria is of serious public health significance. A high degree of hygienic practice is recommended for both buyers and sellers to prevent potential cases of Staphylococcal food poisoning.

Keywords: *Staphylococcus aureus;* Evening markets; Meat

Introduction

Meat from cow (beef) is a major source of protein all over the world. In parts of Nigeria where protein is a short supply [1], cow meat serves as the main source of animal protein for most families. Proper handling of meat poses problems to both. Exposure during sales could result in contamination by a wide range of bacteria. The poor hygienic situation of markets in developing countries like Nigeria could expose meat to bacteria including *Staphylococcus* spp, *Bacillus* spp, *Vibrio* spp, *Salmonella* spp and *Listeria* spp (Wei-wei *et al*., 2018). These organisms affect the quality of meat and are potentially harmful to humans, the consumer.

Staphylococcus aureus is a major foodborne pathogen, growing at temperature 15^{0}C-45^{0}C and NaCl concentration above 15%. [2]. The organism is a facultative anaerobic, Gram positive, non-motile, non-spore forming toxic bacteria [3]. Meat and meat products have been identified as reservoir for *Staphylococcus aureus* [4].

Presently, the genus *Staphylococcus* has 62 species and 30 sub-species of which *Staphylococcus aureus* is the most important foodborne pathogen [5]. Food poisoning could result from the production of heat-stable enterotoxins by *Staphylococcus aureus* [6]. There is a need therefore to improve the quality of meat sold in markets by reducing contact with bacteria and other food spoilage organisms.

Materials and Methods

Study Area

The study was carried out in Yenagoa (4^{0}55'N, 6^{0}16'E), Bayelsa Sate, Nigeria. Samples for the study were collected from three markets;

A. Swali market
B. Opolo market
C. Tombia market

Sample Collection and Preparation

Samples were collected randomly from three sellers from

each of the selected markets. The samples were labelled, packed in polythene bags and transported in ice to the laboratory for preparation and analysis. Collected meat samples were ground using mortar and pestle and 1g of the samples weighed into 9mL of sterile distilled water (1:10 w/v) [7]. This was homogenized using a Stomacher blender. 1ml of the obtained homogenate was measured using a sterile pipette and diluted into 9mL of sterile distilled water (10^{-1}). The serial dilutions were repeated to obtain 10^{-6} dilutions and used for further analysis.

Nutrient agar (Oxoid) and Mannitol Salt agar (Oxoid) used for cultivation of bacteria were prepared as described by Cheesbrough (1985) [8]. Slants were prepared using sterilized specimen bottles.

Sample Inoculation

Prepared aliquots of the sample (0.1 ml) were inoculated onto Nutrient Agar and Mannitol salt agar in duplicates using the spread plated technique [9] and incubated at 37^0C for 24-48 hrs.

Characterization and Isolation

After 24-48 hrs of incubation, the plates were observed for bacterial growth. Colonies were counted, described and recorded. The observed colonies were then transferred to agar slants using wireloop and flame sterilization and incubated at 37^0C for 24 hrs for further observation, identification and subculture on Mannitol Salt agar.

Total Bacterial Count (TBC) was determined using the formula;

$$\text{TBC (CFU/g)} = \frac{\text{No. of colonies}}{\text{Sample volume}} X \text{ Dilution factor}$$

Confirmation of *Staphylococcus aureus*

Standard biochemical tests and molecular techniques were used for the identification and confirmation of *Staphylococcus aureus*. Biochemical tests used included Gram staining, Coagulase test, Catalase test, Methyl red test and Citrate utilization test [10-12].

Molecular Identification

Bacterial isolates sub-cultured on Luria Bertami broth for 24 hrs were subjected to molecular identification using PCR based multiplex assay [13] using target genes that encode for bacterial subunits (16s). The BigDye Terminator kit was used for sequencing and similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using Basic Local Alignment Search Tool Nucleotide (BLASTN) [14]. The evolutionary history was inferred using the Neighbour-Joining method in MEGA 6.0 (Saitou and Nei, 1987) and evolutionary distances were computed using the Jukes-Cantor method [15].

Results and Discussion

Total Bacterial Count (TBC)

After 24 hrs of incubation, the plates were observed for bacterial growth and the total bacterial counts recorded. The results of the total bacterial counts are shown in Table 1. The results indicated that samples from Tombia market had the highest no of bacterial cultures in Colony Forming Units (CFU) while those from Opolo market had the least. The highest total bacterial count of 1.03 x 10^9 CFU/g was recorded for C2 and the least of 1.3 x 10^8 CFU/g was recorded for B1. The results obtained from inoculation on Mannitol salt agar was almost similar.

Sample	Nutrient agar		Mannitol salt agar	
Media	No. of Colonies	CFU/g	No. of Colonies	CFU/g
A1	38	3.80 x 10^8	12	1.2 x 10^8
A2	43	4.3 x 10^8	13	1.3 x 10^8
A3	22	2.2 x 10^8	5	5.0 x 10^7
B1	13	1.3 x 10^8	6	6.0 x 10^7
B2	23	2.3 x 10^8	8	8.0 x 10^7
B3	19	1.9 x 10^8	4	4.0 x 10^7
C1	62	6.2 x 10^8	29	2.9 x 10^8
C2	103	1.03 x 10^9	15	1.5 x 10^8
C3	91	9.1 x 10^8	16	1.6 x10^8

Table 1: Total bacterial counts (TBC) on Nutrient agar and Mannitol salt agar. CFU/g: Colony forming units per gram.

Table 2 shows a comparison of the number of bacterial colonies obtained from Nutrient agar and Mannitol salt agar. The colony counts on Nutrient agar were far more than that on Mannitol salt agar. Mannitol salt agar is a selective media. The correlation coefficient using Pearson's correlation coefficient showed that the results were significant at p(0.05) with $r_{(cal)}$ (0.529)<$r_{(tab)}$(0.666)

Nutrient agar	Mannitol salt agar
38	12
43	13
22	5
13	6
23	8
19	4
62	29
103	15
91	16

Table 2: Comparison of Colony count from media.

Morphological Characteristics of Bacteria

The morphological description of the cultures observed after incubation on Nutrient agar are presented in Table 3. The morphological characterization of the observed cultures indicates the growth of five types of bacteria on Nutrient agar. R1 showed golden-yellow colonies typical of *Staphylococcus aureus*. R2 were white, discrete, pancake colonies typical of species of *Streptococcus* spp. R3 were colonies that are white and serrated typical of species of *Bacillus* spp. R4 had white, flat colonies that are generally representative of *Micrococcus* spp and R5 showed white, shiny colonies that could indicate *Enterococcus* spp.

Isolate	Morphological description	Probable identity
R1	Large, golden-yellow	*Staphylococcu aureus*
R2	White, pancake-like	*Streptococcus* spp
R3	White, serrated	*Bacillus* Spp
R4	White, flat	*Micrococcus*
R5	White, shiny	*Enterococcus*

Table 3: Morphological description of bacterial cultures on Nutrient agar.

The results of the bacterial isolates sub-cultured on Mannitol salt agar are presented in Table 4. Results showed the growth of the isolates on the selective media with neutral red indicator. R1 showed the ability to ferment mannitol. This is a confirmation of *Staphylococcus aureus*. R4 showed growth with no colour change indicating *Staphylococcus* spp. unable to ferment mannitol typical of *Staphylococcus epidermidis*. R3, R4 and R5 had inhibited growth.

Isolate	Morphological description	Probable identity
R1	Yellow colour change	*Staphylococcu aureus*
R2	Inhibited growth	*Streptococcus* spp
R3	Inhibited growth	*Bacillus* Spp
R4	No colour change	*Staphylococcus epidemidis*
R5	Inhibited growth	*Enterobacter* spp

Table 4: Morphological description of isolates sub-cultured on Mannitol salt agar.

Biochemical Characterization of Bacteria

The results obtained from the biochemical identification of the isolated bacteria are presented in Table 5. Results from Gram staining and observation with the oil immersion lens indicated that R1, R2 and R4 are Gram positive cocci in clusters while R3 are Gram positive rods and R5 are Gram negative rods.

Isolate	Gram's reaction	Coagulase test	Catalase test	MR test	Citrate utilization	Probable organism
R1	+	+	+	+	+	*Staphylococcus aureus*
R2	+	-	+	+	+	*Streptococcus* spp
R3	+	-	-	-	+	*Bacillus* spp

R4	+	+	+	+	+	*Staphylococcus aureus*
R5	-	-	+	-	+	*Enterobacter* spp

Table 5: Biochemical characterization of isolated bacteria.

For the coagulase test for the presence of the enzyme *Coagulase*, the results showed that R1 and R4 are coagulase positive while the others (R2, R3 and R5) are coagulase negative i.e. they do not possess coagulase.

The catalase test showed that four of the isolates (R1, R2, R4 and R5) are catalase positive while one (R3) is catalase negative. Catalase breakdown hydrogen peroxide into hydrogen gas and water vapour (effervescence observed). The combination of the results of the various biochemical tests confirm R1 and R4 as *Staphylococcus aureus*, which are Gram positive, coagulase positive and catalase positive.

Molecular Identification of Bacteria

Uploading the DNA sequence of the extracted and amplified 16s DNA for R1 and R4 to the NCBI database produced a match. Both had a 100% match with *Staphylococcus aureus* strain UA899. The BLAST technique compared the 16s DNA sequence obtained for the isolated sample with similar sequence found in the database. The Neighbor-joining method was used to infer the evolutionary history of the isolated organism.

Conclusion

The results obtained from the study indicate that meat obtained from evening markets within Yenagoa had significant incidence of *Staphylococcus aureus*. Significant amount of the bacteria in the selected samples is of public health significance due to the ability of the organism to cause a wide range of infections. Higher levels of the bacteria in samples obtained from Tombia market is indicative of the socio-economic activities within the vicinity. Occurrence of *Staphylococcus aureus* in the samples may be due to contact with anthropogenic sources. Exposure of meat to the surrounding environment from the time of slaughter in the morning to the evening when they are sold will result in significant exposure to the bacteria [16].

Non-hygienic practices by buyers, sellers and passers-by could also result in exposure to the bacteria which is a normal flora of humans inhabiting the skin and the nasal tract. Recent evidence has shown that *Staphylococcus aureus* develops antibiotic resistance [6,17] and this could result in the presence of toxigenic strains of the bacteria in food that could result in serious public health consequences.

The presence of the bacteria in the samples may not indicate immediate health risks since meat and meat products are cooked before consumption. Occurrence of *Staphylococcus aureus* in meat and meat products was also reported by [16,18]. However, the presence of Coagulase positive strains calls for caution. There have been reported cases of Staphylococcal food poisoning in cooked food, preserved food and Ready-to-Eat food [19]. Food safety begins with production and continues to handling and finally consumption.

Sellers of meat and meat products should adopt better hygienic practices to ensure food safety. Meat bought from markets should be thoroughly cleaned, washed and cooked before consumption to prevent incidence of Staphylococcal food poisoning. The use of specially designed table and sheds with nets for the sale of meat and meat products to prevent unnecessary exposure of the products. Further studies on the implication of the bacteria in the selected areas should be carried out.

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