Staphylococcus species Isolated from Abattoir in Obio/Akpor and Eleme Local Government Areas of Rivers State

Abstract

Abattoir operation could be very beneficial to man; in that it provides meat for human consumption and other by-products.The study was aimed at isolation of *Staphylococcus* species from various equipment used in abattoir. A total of 100 samples were collected from five different surfaces; bucket swab, butchers hand swab, table swab, knife swab and meat swab from four abattoirs; two from Obio/Akpor and two from Eleme Local Government of Rivers State. The samples were transported aseptically to laboratory for immediate analysis. The samples were cultured using standard microbial method on Mannitol salt agar. The isolates of *Staphylococcus* species were identified using biochemical tests. The *Staphylococcal* counts ranged from 10.64±0.07 to 25.50±0.71×10^5 CFU/g for bucket. The least count for the bucket sample was observed in Akpajo while the highest count was observed in Rumuokurushi. For the meat samples the counts ranged between 8.40±0.14 to 12.85±0.07 ×10^5 CFU/g, the least count was observed in Rumuokoro, while the highest was observed in Aleto. For the hand, knife and table samples the least counts of 10.35±0.35 ×10^5 CFU/g, 12.50±0.14 ×10^5 CFU/g and 14.80±0.14 ×10^5CFU/g were observed from Akpajo, and Rumuokoro, respectively, while the highest count was obtained from hand (26.75±0.07 ×10^5CFU/g), knife (15.30±0.14x10^5CFU/g) and table (22.50 ± 0.71x10^5 CFU/g) in Aleto, Rumuokrushi and Akpajo, respectively. A total of 43 isolates of *Staphylococcus* species was obtained in the study of which least percentage occurrence of 2.33% was record from table in Akpajo while the highest percentage occurrence of 9.3% was recorded from bucket in Akpajor. The results shows that cross-contamination of meat sold in the abattoir could come from the equipment used in the abattoir hence, their hygienic condition should be ensured.

Keyword:Bucket swab, butcher hand swab, table swab, knife swab, meat swab

1.0 Introduction

An abattoir or slaughterhouse is referred to as a facility where animals are slaughtered for consumption as food products. Abattoir operation could be very beneficial to man; in that it provides meat for human consumption and other by-products. Also, it can be hazardous to public health if the waste it generates is not properly disposed (Adeyemi and Adeyemo, 2007). It produces highly characteristic organic waste with relatively high level of suspended solids, liquids and fats. These include condemned meat, undigested bones, hairs, horns, aborted foetuses, gut contents, blood, urine and water (Adeyemo *et al*., 2002).Equipment such as tables, hooks and machines, etc. are used in the abattoir for different purpose, this equipment should be made of non-corrosive materials. Essential for the hygienic handling of carcasses and meat is equipment for hoisting the carcasses, when slaughtered. Food security is more critical than food safety among both rural and urban poor. The General Slaughter process includes inspection and grading of carcass by a Veterinary inspector for quality and safety. Carcass is cut apart and the body parts separated (Tekki *et al*., 2012). In a standard abattoir, meat cuts are quickly chilled to

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prevent the growth of microorganisms and to reduce meat deterioration while the meat awaits market demand for distribution. The remaining carcass may be further processed to extract any residual traces of meat while materials not destined for human consumption is sent to a rendering plant, and the meat is finally transported to local retail markets (Tekki *et al*., 2012).

Despite availability of legislature and involvement of trained personnel in some cases, myriads of problems abound with meat handling procedures in most abattoirs of Nigeria. These problems range from: Slaughtering on the floor, absence of stunning and ripening operations, inadequate slaughtering facilities, lack of sewage disposal systems, inadequate clean water supply, lack of a refrigeration system, lack of adequate transport system for meat products to lack of price incentives for quality (Gail, 1997). The Staphylococci are well known as bacterial pathogens causing multiple types of infections in both human and animals (Le Loir *et al*., 2003; Salasia *et al*., 2004). Staphylococcus aureus is one of the foods borne diseases transmitted from the contaminated animal source food staffs (Nouichi and Hamdi, 2009). It produces heat stable and proteolytic enzyme resistant enterotoxins that cause food poisoning in humans leading to vomiting, abdominal pain and diarrhea (Busani *et al*., 2006).

These organisms can be transmitted to humans who consume contaminated meat obtained from abattoir. This could be the sole source of contamination in abattoir and butcher workers for those who do not have enough awareness on the nature of the disease. This study is aimed at isolation of *Staphylococcus*species from various equipment used in abattoir.

2.0 Materials and Methods

2.1 Sample Area and Collection

The sampling was conducted in four abattoirs; two from Obio/Akpor and two from Eleme Local Government of Rivers State and written authorization from the butchers was obtained. Samples were obtained from five different surfaces: Bucket swab, butchers hand swab, table swab, knife swab, meat swab. All experiments were performed in duplicate under aseptic conditions. The sterile swabs were soaked in peptone solution, and rubbed on the surface. After the friction swabs were placed individually into previously identified tubes containing 10 ml of sterile peptone solution, and put in icebox cooler and taken to the laboratory for further study (Lara *et al*., 2019).

2.2 Serial dilution and Culturing

Serial dilution was carried out by dispensing 9ml of already prepared normal saline into three test tubes labeled 10^{-1}- 10^{-3} and autoclaved at 121^{o}C for 15 min at a pressure of 15psi. After cooling, the samples were inoculated into the first test tube labeled 10^{-1} and properly mixed. A sterile pipette was used to pipette 1ml of mixture is taken from the 10^{-1} dilution and dispensed into the second tube to dilution of 10^{-2}. The same process was then repeated for the remaining tube, taking 1ml from the previous tube and adding it to the next 9 ml diluents.*Staphylococcus* species were enumerated byspread plate method and grown on

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Mannitol Salt Agar (MSA). An aliquot (0.1 mL) from the appropriate dilution was inoculated into pre-dried MSA plates. The inoculated plates were incubated at 37°C for 24 hrs. After incubation, yellow colonies were counted and recorded aspathogenic *Staphylococci* counts (APHA, 2012).

Based on the different morphological characteristics, the different isolates were purified by picking specific colonies with a sterilized inoculating loop and spread on freshly prepared nutrient agar. This was repeated for all the isolates on each prepared medium. The inoculated media plates were incubated at 35±2°C for 24-28 hrs.

2.3 Bacterial Identification of the Isolates

Gram stain, catalase and sugar fermentation tests were used in the identification of the bacterial isolates from the samples(Amenu, 2014).

2.3.1 Gram Staining

A clean, grease free slide, was used to prepare a smear of suspension with a loopful of sample. It was allowed to air dry followed by heat fixing. Crystal Violet was poured on the slide and left for 1 min and rinsed with water at angle 45^{o}C. The slide was again flooded with Gram's iodine for 1 min and wash off with water. After which, 95% alcohol was used to decolorizing about 10-20 sec and rinse with water. Finally, safranin was used to cover the slide for about 1 min and wash with water. The slide was then allowed to air-dry, and observe using oil immersion under the microscope.Colonies that showed blue/purple cocci bacteria were selected.

2.3.2 Sugar Fermentation Test

2.3.2.1 Mannitol Test

The purpose of mannitol fermentation testwas to determinethe microbes that can ferment thesugar, mannitol as a carbon source. Phenol red mannitol broth medium containing 0.5-1.0% mannitol was prepared.Phenol red is red which is a pH indicator turns yellow at pH <6.8. It also changes to magenta or hot pink at pH >8.4. An inoculum from a pure culture of the isolates was transferred aseptically to a sterile test tube of phenol red mannitol broth. The inoculated tube was incubated at 35-37^{o}C for 24 hrs and the results were determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic(Amenu, 2014).

2.3.2.2 Sucrose Test

An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol redbroth containing 0.5-1% sucrose. The inoculated tube was incubated at 35-37 ^{o}C for 24 hrs and the results were determined by observing the color change. A positive test consists of a color change from red to yellow, indicating a pH change to acidic(Amenu, 2014).

2.3.2.3 Glucose Test

An inoculum from a pure culture of each of the isolates was transferred aseptically to a sterile tube of phenol red glucose brothcontaining 0.5-1% glucose sugar. The inoculated tube was incubated at 35-37^{o}C for 24 hrs and the results were determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic(Amenu, 2014).

3.0 RESULTS

Results from the *Staphylococcal* counts ranged between 10.64±0.07 to 25.50±0.71×10^5 CFU/g for bucket. The least count in the bucket was observed in Akpajo while the highest count was observed in Rumuokurushi. For the meat samples the counts ranged between 8.40±0.14 to 12.85±0.07 ×10^5 CFU/g, the least count was observed in Rumuokoro, while the highest was observed in Aleto. For the hand, knife and table samples the least counts of 10.35±0.35 ×10^5 CFU/g, 12.50±0.14 ×10^5 CFU/g and 14.80±0.14 ×10^5CFU/g were observed from Akpajo, and Rumuokoro, respectively, while the highest count was obtained from hand (26.75±0.07 ×10^5CFU/g),knife (15.30±0.14x10^5CFU/g) and table (22.50 ± 0.71x10^5 CFU/g) in Aleto, Rumuokrushi and Akpajo, respectively (Table 1). A total of 43*Staphylococcus* spp. were isolated from the Abattoirs in the different locations. Figure 1 represents the frequency of occurrence of *Staphylococcus*spp.in the various abattoir.The table recorded least percentage occurrence of 2.33% In Akpajo while the highest percentage occurrence of 9.3% was recorded from bucket in Akpajo.

Table 1: Mean *Staphylococcal* Count isolated from the Abattoir

Location	Bucket ×10^5 Cfu/g	Meat ×10^5 Cfu/g	Hand ×10^5 Cfu/g	Knife ×10^5 Cfu/g	Table ×10^5 Cfu/g
Akpajo	10.64±0.07^a	8.65±0.21^a	10.35±0.35^a	13.25±0.21^c	22.50±0.71^c
Aleto	19.50±0.70^b	12.85±0.07^c	26.75±0.07^d	13.50±0.71^b	15.50±0.71^a
Rumuokoro	19.79±0.14^b	8.40±0.14^a	16.20±0.14^b	12.50±0.14^b	14.80±0.14^a
Rumuokrushi	25.50±0.71^c	9.80±0.14^b	18.60±0.14^c	15.30±0.14^c	20.65±0.21^b

*Mean with the same superscript along the columns is not significantly different (P≥0.05)

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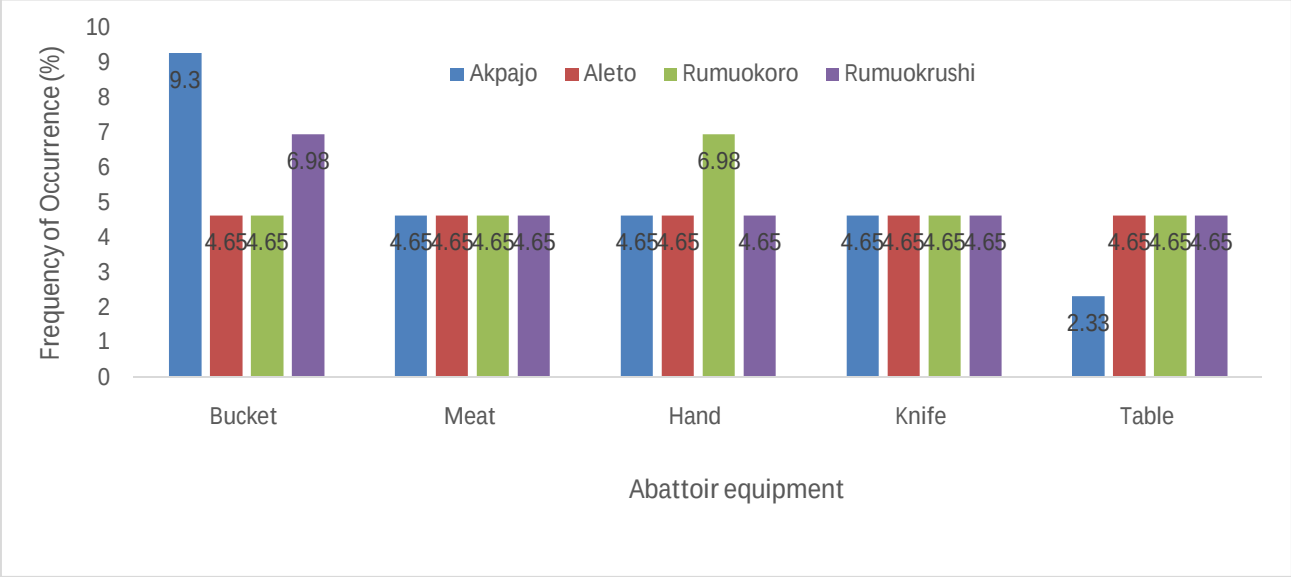

Fig. 1: **Frequency of occurrence of *Staphylococcus*spp. from the different Abattoir**

Table 2 shows the glucose analysis of the isolates in all the locations. In the glucose, acid producing isolate recorded the highest percentage in Rumuokwurushi (20.930%) while the least percentage was recorded in Akpajor, Aleto and Rumuokoro. In the glucose analysis for Acid and gas the highest percentage was recorded in Akpajo, Aleto and Rumuokoro and Rumuokwurushi (4.651%) and none was recorded in Aleto. For the glucose negative test, the highest percentage was recorded in Aleto (6.977%) and the least in Akpajor and Rumuokoro.

Table 3 shows the sucrose analysis of the isolates from all the sample locations. The highest percentage for sucrose acid was recorded in Rumuokwurushi (13.953%), while the lowest were recorded in Akpajor, Aleto and Rumuokoro abattoir (11.628%), for sucrose analysis for acid and gas the highest percentage was also recorded in Rumuokwurushi (9.302%) while the least was found in Aleto. Sucrose negative result, the highest percentage was recorded in Akpajor, Aleto and Rumuokoro (9.302%) while the least was recorded in Rumuokwurushi (2.326%).

Table 4 shows the mannitol analysis of the isolates from all the sample locations. The highest percentage for mannitol acid was recorded in Akpajor (13.953%) while the least was recorded in Aleto (4.651%). For the mannitol acid and gas, the least was recorded on Akpajor (2.326%). For mannitol negative, the highest percentage was recorded in Aleto (18.605%) while the least was recorded in Rumuokwurushi and Akpajor abattoir (9.302%).

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Table 2:Glucose analysis of the isolate

Abattoir location	Glucose-A (%)	Glucose-AG (%)	Glucose-Negative (%)
Akpajor	7(16.279)	2(4.651)	2(4.651)
Aleto	7(16.279)	0(0.000)	3(6.977)
Rumuokoro	7(16.279)	2(4.651)	2(4.651)
Rumuokwurushi	9(20.930)	2(4.651)	0(0.000)

Table 3: Sucrose analysis of the isolate

Abattoir location-	Sucrose-A (%)	Sucrose-AG (%)	Sucrose-Negative (%)
Akpajor	5(11.628)	2(4.651)	4(9.302)
Aleto	5(11.628)	1(2.326)	4(9.302)
Rumuokoro	5(11.628)	2(4.651)	4(9.302)
Rumuokwurushi	6(13.953)	4(9.302)	1(2.326)

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Table 4: Mannitol analysis of the isolate

Abattoir location	Mannitol-A (%)	Mannitol-AG (%)	Mannitol-Negative (%)
Akpajor	6(13.953)	1(2.326)	4(9.302)
Aleto	2(4.651)	0(0.000)	8(18.605)
Rumuokoro	3(6.977)	2(4.651)	6(13.953)
Rumuokwurushi	3(6.977)	4(9.302)	4(9.302)

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4.0 Discussion

Staphylococcus species is one of the most prevalent causes of foodborne and clinical infection globally and hence has attracted public attention due to the increasing mortality associated with their multidrug resistance (Tsepo *et al.*, 2016). Some species of *Staphylococcus* have been reported for their implication in the cause of enteric diseases especially in developing countries (Balaban and Rasooly, 2000). Across the four locations sampled, there was different ranges of *Staphylococcal* count however, it was observed that higher count of *Staphylococcus* was recorded in materials in the abattoir bucket, knife and table and hand (skin) of the butchers compared to the meat samples analysed for all the sampled collections.The higher microbial enumeration recorded in the sampled materials such as buckets, knife, hand and table can be understood to be the major source of the bacterial contamination in meat sold in the abattoir. Consumers of meat bought from the study locations could be at risk of having*Staphylococcus* food poisoning symptoms which are characterized by nausea, vomiting, diarrhea, general malaise and weakness,within 24 h of ingestion (Thawla *et al.*, 2021).*Staphylococcus* species is a commensal of the human nasopharynx, mouth and the skin. It can be present in the soil, water, dust and air especially in a populated environment. The presence of species of *Staphylococcus* can also be attributed to or connected to unhygienic or improper handling from the butchers and from the environments (Tsepo *et al.*, 2016). During talking, coughing, touching, sneezing, laughing, species of *Staphylococcus* can be introduced to meat in the abattoirs (Yeh, 2004).Contamination with *Staphylococcus* is crucial in the evaluation of the safety and hygienic quality of meat, and also in determining the origin of food poisoning in abattoir (Karmi 2013).Highest percentage occurrence (9.3%) of the genera, *Staphylococcus* were observed in the bucket in Akpajo, which is lower than the record in the study ofAdugna *et al*. (2018) andthat of Samutela *et al*. (2022),however is in line with similar studies on the African continent that have reported prevalence ranging from 0% to 55% (Samutela *et al.*, 2021).The higher occurrence of

Staphylococcus could point to the major source of possible contamination by the bacteria,*Staphylococcus* in meat in abattoir. From this study, buckets, knife, table used in the abattoir can be said to the major source of *Staphylococcus* in the abattoir. Poor maintenance of hygienic condition of these materials used might have resulted to the higher prevalence of the bacteria (Adugna *et al.*, 2018). The fermentation of sugars and gas production are among the important features of bacteria of the *Staphylococcusaureus*. Most isolates of *Staphylococcus aureus* have the ability to produce acid from various sugar types, such as glucose, mannitol, mannose, terhalose, maltose and sucrose. (Amini *et al.*, 2013). The difference in percentage in the fermentation varies among different strains and species of the microorganisms and the source of the organisms (Amini *et al.*, 2013).All thepercentage fermentation of the sugars recorded in this study was less than that recorded in the study of Verma *et al.* (2022) in which percentage fermentation of 88.9%, 98.83% and 84.21% was recorded for glucose, Mannitol and Sucrose respectively by isolates of *Staphylococcus* from farm animals. The difference in the percentage fermentation and gas production of these sugars could be related to the difference in the strains and the source of the samples.Amini *et al* (2013) published a research article on comparison of Ferment Sugars in Methicillin-Resistant and Methicillin Sensitive *Staphylococcus aureus* isolates and they found thatglucose was fermented by all *Staphylococcus aureus* isolates during 24h compared to other sugars.

Conclusion

The presence of *Staphylococcus* species in food such as meat is of great concern. From this study, it can be concluded that the major sources of contamination of meant by *Staphylococcus* species in abattoir is materials used in abattoir such as buckets, knife, tables and hand of butchers. The possible contamination by these materials can be linked to the unhygienic state of these abattoir and the handling thus result in cross contamination in these abattoirs. It could be recommended that activities in the abattoir should be monitored and regulated by the government and sanitation agencies to ensure that approved and acceptable standards are adhered to reduce contamination of meat that get to final consumers.

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