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BACTERIAL PATHOGENS ISOLATED FROM *PSEUDOTOLITHUS SENEGALENSIS* AND *MICROMESISTUS POUTASOU* SOLD IN SOME MARKETS IN LAGOS STATE

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ABSTRACT

The bacterial qualities of two smoked fish species *Pseudotolithus senegalensis* (croaker) and Blue whiting, *Micromesistus poutasou* (panla) sold in four different fish markets in Lagos, Nigeria were examined. Standard aerobic pour plate method was used for the isolation of bacteria. For *Pseudotolithus senegalensis*, the highest bacterial count (5.23 Log_{10} cfu/g) was observed in samples purchased from Iyana Ipaja market while for *Micromesistus poutasou*, the highest bacterial count (5.08 Log_{10} cfu/g) was observed in samples purchased from Liverpool market. Bacteria of the family Enterobacteriaceae were isolated from samples purchased from the four markets. The presence of these organisms shows that these samples have been contaminated and could pose significant public health risks. Therefore, it is important that fish smoking should be carried out under hygienic conditions and cooking of smoked fish adequately before consumption is highly recommended so as to avoid food borne diseases.

Keywords: *Pseudotolithus senegalensis*, *Micromesistus poutasou*, Bacterial count, Smoked fish.

INTRODUCTION

Fish is an important source of nutrients to man, because of its high protein content, low carbohydrate and fat content (Sarojnalini and Hei, 2019). It is an essential source of animal protein in developing countries and it represents about 14% of all animal proteins on a global basis (Abolagba and Melle, 2008; Mohanty *et al.*, 2019). It is extremely perishable. In Nigeria, the quality of the fish could be affected by warm climatic conditions, unhygienic environment and improper handling. Fish is highly perishable after catch and thus should be preserved quickly after catch to retain its quality. Preservation techniques used in Nigeria include salting, smoking and freezing among others (Adeyeye and Oyewole, 2016) with smoking being the most common. In Nigeria, smoking is one of the oldest methods of processing fish and remains inexpensive in less developed countries (Agu *et al.*, 2013). It has been reported that smoking of fish and fish products are greatly practiced and an acceptable method of preservation where advanced equipment for better methods are inadequate. Fish smoking can be described as the lowering of the water activity through the application of low heat. The surface of the fish skin, which is vulnerable to the growth of microorganisms, is dried while the heat and the chemicals basically in the smoke prevents the microorganisms from growing (Fahim *et al*., 2017). The brilliant color and improved flavor of smoked fish makes it desirable and acts as a safeguard against enzymatic, microbiological and chemical deteriorative alterations (Akinwumi and Adegbehingbe, 2015). Smoked fish contains numerous nutritional elements that have good health benefits. Thus in Nigeria, there is a widespread consumption of fish by both low and middle level groups. Although, seafoods may be considered as safe and healthy, any occurrence of bacterial pathogens in them may be an indication of insufficient food safety (Mossel *et al.*, 1995).

Fish and fishery products generate employment and revenue; however, it can be a source of bacterial hazards including *Staphylococcus aureus*, *Salmonella* spp and *Clostridium botulinum* (Olaleye and Abegunde, 2015). Fish are easily affected by a variety of bacterial pathogens such as *E. coli*, coagulase-positive *Staphylococcus*, *Aeromonas* and *Vibrio* spp. Poor food safety practices leads to the consumption of inadequately processed fish or fishery products that are contaminated during/after their processing which often results in sudden occurrence of an illness or disease (Uchedu, 2018). In a developing country like Nigeria, smoked fish are often in contaminated environment. Microorganisms could contaminate fish products, either from the processing units or market centers before getting to the consumers (Akinwumi and Adegbehingbe, 2015). The presence of pathogens does not only show the unhygienic condition of the processing environment, but also possibility of pathogenicity to the consumer. Therefore, this research is aimed at determining the bacterial pathogens associated with smoked fish from some markets in Lagos.

MATERIALS AND METHODS

Study area

The fish samples were purchased from four markets in Lagos State namely: Badagry, Iyana Ipaja, Liverpool and Mushin. Lagos is known to be the most populous city in the whole of Africa with two sea ports and it serves as a trade portal, not only to Nigeria, but to other West African countries (Jibiri *et al.*, 2014).

Sample collection

Two different fish species namely *Pseudotolithus senegalensis* (croaker) (59 samples) and *Micromesistus poutasou* (panla) (55 samples) were purchased from four markets in Lagos metropolis namely: Badagry (croaker (cr)-18, panla (pa)-15); Iyana Ipaja (cr-12, pa-12); Liverpool (cr-15, pa-14) and Mushin (cr-14, pa-14). The smoked fishes are displayed in baskets or plastic basins (Ayo-Olalusi *et al.*, 2010). Sample collection was carried out on a monthly basis from January – December 2016. The samples were purchased in the morning, wrapped in newly purchased polyethylene bags and labeled accordingly. These samples were transported to the Microbiology laboratory of the Nigerian Institute for Oceanography and Marine Research for identification and bacteriological analysis. The bacteriological assessment of smoked fish was analyzed using standard microbiological methods according to APHA (1997).

Bacteriological analysis

Sterile forceps was used to cut the fish. Ten (10) grams of the fish tissue was cut out and weighed using a weighing balance. Each of the weighed sample was transferred to a conical flask containing 90mls of sterile distilled water to make the stock suspension, which is the 10^{-1} dilution. One (1) ml of the stock was serially transferred to five test tubes each containing 9mls diluent, to obtain 10^{-6} dilution.

Dilutions of 10^{-2}, 10^{-4}, 10^{-5} were selected and plated using the pour plate method. For the isolation of *Staphylococus* spp, Mannitol salt agar was used. Eosin methylene blue agar was used to isolate *E. coli*, while *Salmonella- Shigella* media was used to isolate *Salmonella* spp. This was duplicated and incubated at 37^{o}C for 24hrs. After incubation, the colonies were counted using a colony counter. The numbers were expressed in colony forming units per gram (cfu/g) and later converted to $\log_{10}$.

Statistical analysis

The total viable count expressed in colony forming units per gram (cfu/g) was converted to natural log before statistical analysis. The means were calculated using Microsoft excel (2010).

RESULTS

The bacterial load of *P. senegalensis* and *M. poutasou* are shown in figure 1. It reveals that for *P. senegalensis*, the mean values ranged from 4.3 – 5.23 $\log_{10}$ cfu/g while *M. poutasou* ranged from 4.06 – 5.08 $\log_{10}$ cfu/g. It also shows that, for *P. senegalensis*, the highest total viable count was found in samples obtained from Iyana Ipaja (5.23 $\log_{10}$ cfu/g) and the lowest total viable count was observed at Liverpool (4.3 $\log_{10}$ cfu/g) while for *M. poutasou*, the lowest total viable count was found in samples obtained from Badagry (4.06 $\log_{10}$ cfu/g) while the highest total viable count was observed at Liverpool (5.08 $\log_{10}$ cfu/g).

For *P. senegalensis*, Badagry and Iyana Ipaja (66.7%) recorded a high incidence rate of fish samples infected with bacterial pathogens, obtained from the retail markets while Mushin recorded the lowest (42.85%). However, for *M. poutasou*, Liverpool recorded the highest incidence rate (92.8%) while Mushin recorded the lowest as well. This is shown in figure 2.

Bacterial isolates of the family Enteriobacteriaceae (*Staphylococcus aureus*, *Salmonella* spp and *Escherichia coli*) were found in the fish samples under this study. This is shown in figure 3 and 4. For smoked *P. senegalensis*, *Staphylococcus aureus* was isolated in all the samples obtained from the four markets. *Salmonella* spp was not found in samples obtained from Badagry and Mushin while *Escherichia coli* was not found in samples obtained from Badagry. In the case of smoked *M. poutasou*, *S. aureus* was isolated in all the samples obtained from the four markets as well, while *Salmonella* spp and *E. coli* were not found in samples obtained from Badagry and Iyana Ipaja.

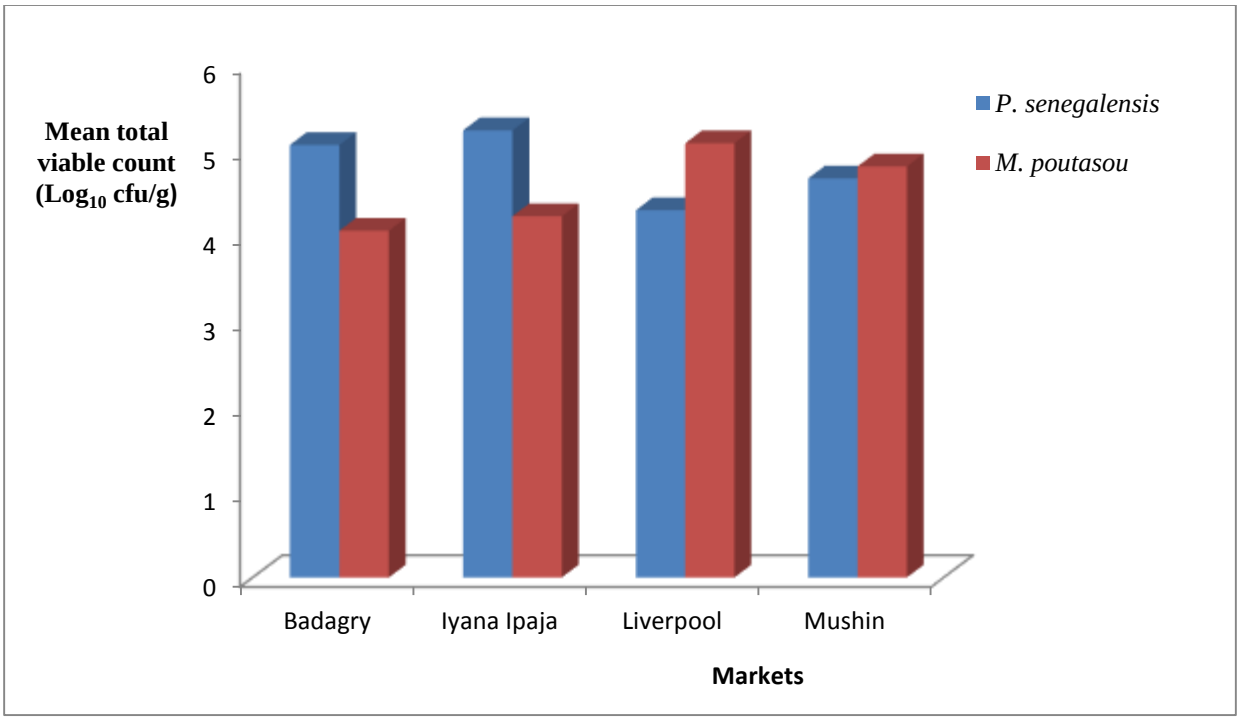

Fig. 1: Mean total viable count of smoked *P. senegalensis* and *M. poutasou* in retail markets in Lagos state.

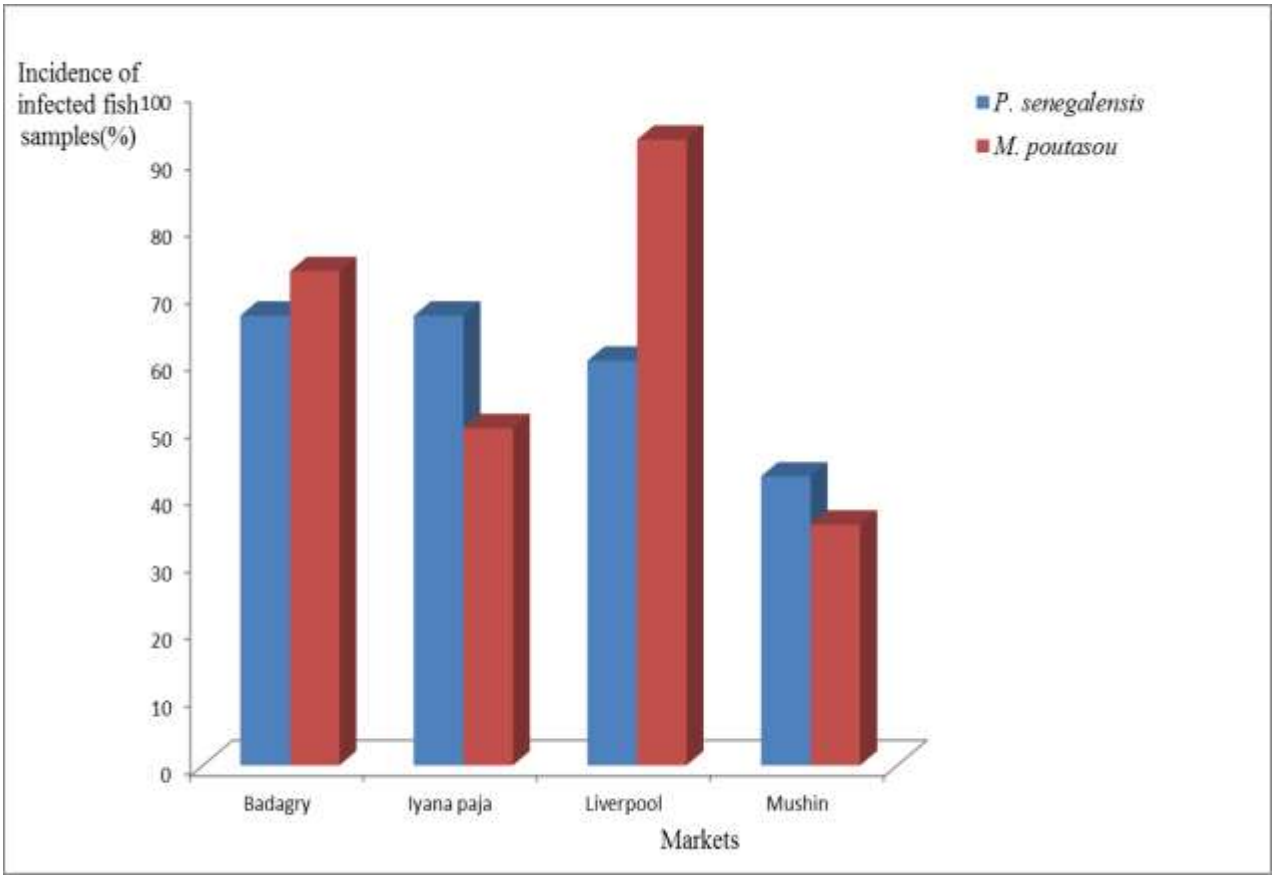

Fig. 2: Incidence of infected fish samples obtained from the retail markets.

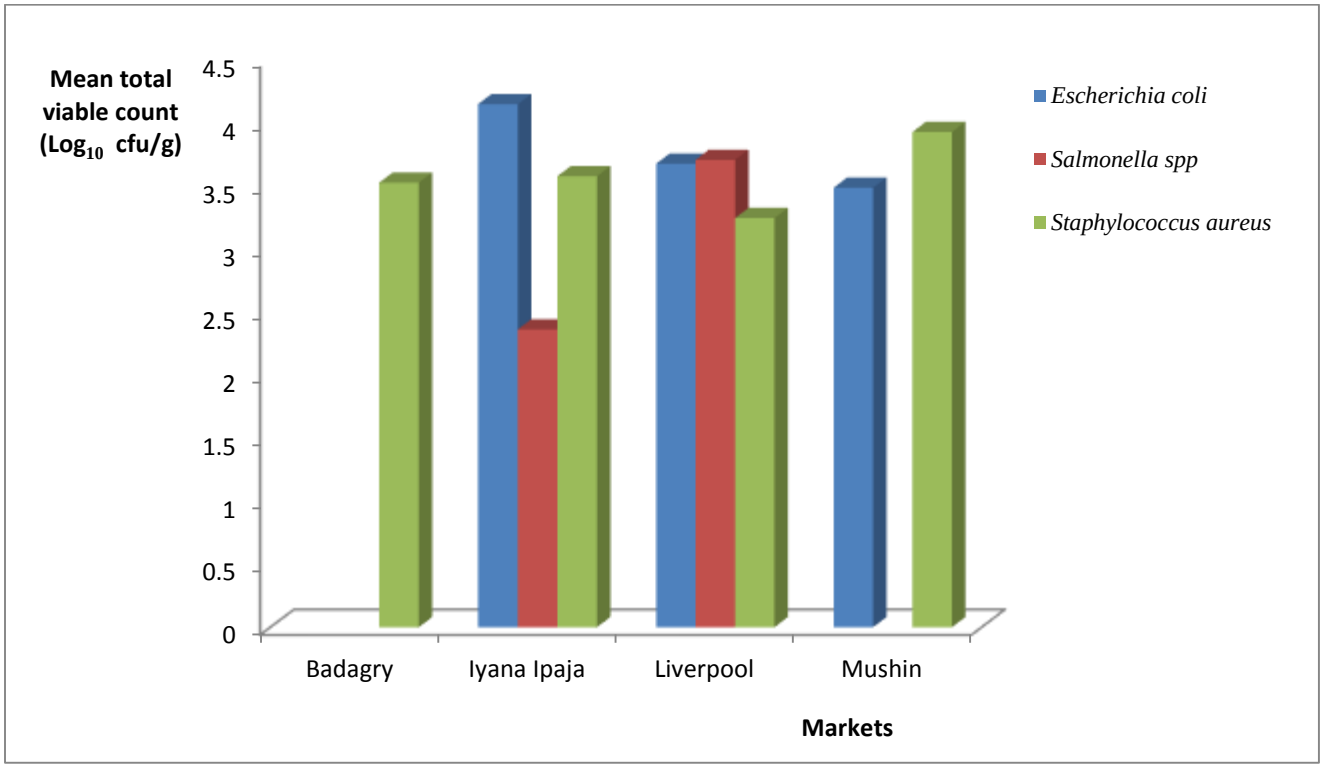

Fig. 3: Bacteriological assessment of smoked *P. senegalensis* in retail markets in Lagos State.

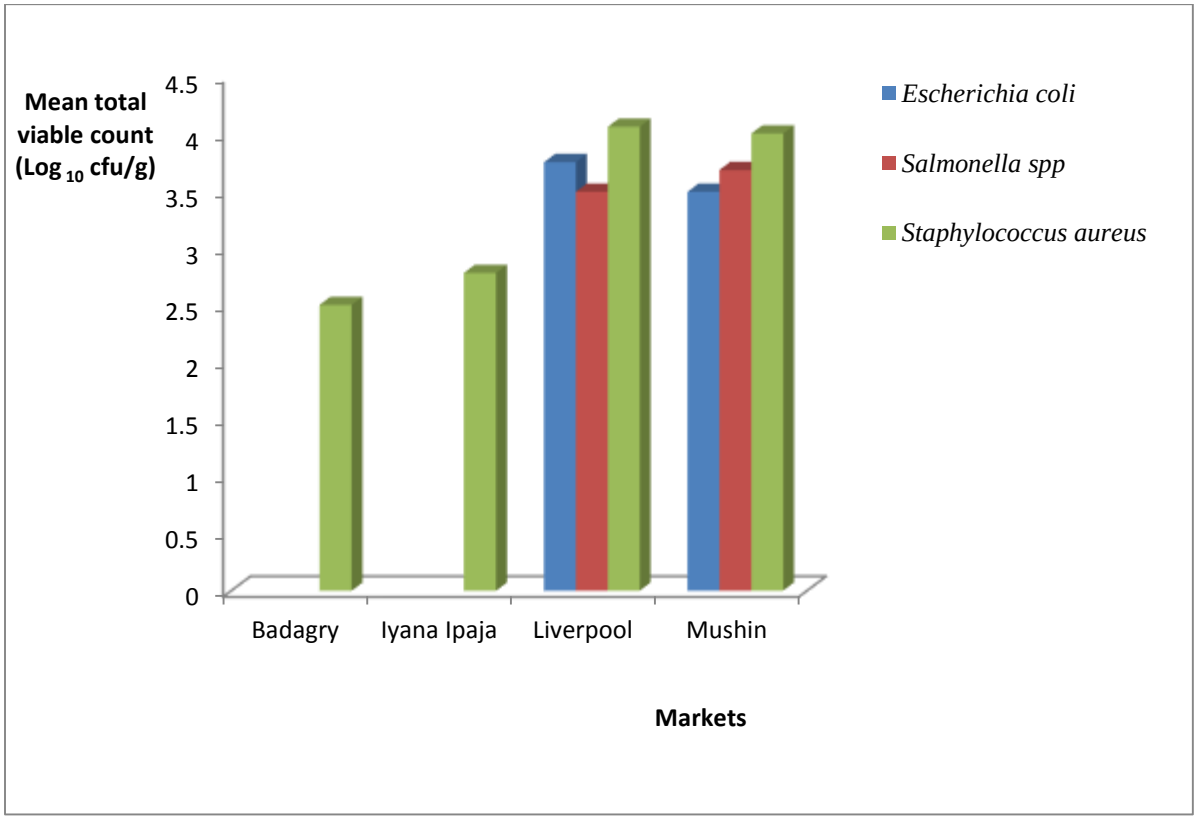

Fig. 4: Bacteriological assessment of smoked *M. poutasou* in retail markets in Lagos State.

DISCUSSION

In the assessment of bacterial quality in fish, the total viable count (TVC) is an important factor and it indicates the general extent of bacterial contamination of foods (International Commission on Microbiological Specification for Foods, 1996). As observed in the study, the TVC revealed that for smoked *P. senegalensis* (croaker), it ranged between 4.3 Log_{10} cfu/g and 5.23 Log_{10} cfu/g, and smoked *M. poutasou* (panla) was between 4.06 Log_{10} cfu/g and 5.08 Log_{10} cfu/g. This is quite different from the findings of other researchers. Daniel *et al*. (2013) reported that the TVC of *P. senegalensis* (croaker) was 1.38 x 10^6 (6.13 Log_{10} cfu/g). The differences in bacterial counts of smoked fish from the various markets can be attributed to improper smoking, unhygienic and handling methods adopted by the fishmongers. Abolagba and Iyeru (1998) reported that improper smoking and mishandling of smoked fish products could be as a result of variations in bacterial load. Amusan *et al*. (2010) also reported that the total viable count for smoked *M. poutasou* (panla) was between 2.4 x 10^3 (3.38 Log_{10} cfu/g) and 2.9 x 10^6 (6.46 Log_{10} cfu/g). The bacterial load for smoked *P. senegalensis* (croaker) and smoked *M. poutasou* (panla) was found to be higher at Iyana paja and Liverpool respectively and this might be due to poor hygiene, secondary contamination and poor sanitary conditions during processing. It is interesting to note that fish harbors many microorganisms. This is one of the factors that contribute majorly to poor quality of fish and in the retail markets, the unhygienic conditions, improper handling and poor storage facilities leads to spoilage as a result of physical damage and contamination with microorganisms (Fahim *et al*., 2017). It has also been reported by Eyo (2001) that in the spoilage of fish, bacterial action plays a major role. Spoilage of fish by microorganisms is often characterized by offensive odors, slime production and softening of the muscle tissue.

As a matter of fact, smoking helps to inhibit the growth of microorganisms however, when this is not done properly, bacterial activities persists leading to spoilage of the fish. This study also revealed that bacterial pathogens like *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were isolated from the fish samples. For *P. senegalensis*, *E. coli* was not found in samples obtained from Badagry, while *Salmonella* spp was not found in samples obtained from Mushin. As in the case of *M. poutasou*, these bacterial isolates were not found in samples obtained from Badagry and Iyana Ipaja. This is also similar to the findings of Amusan *et al.* (2010) who reported that *E. coli* and *Salmonella* spp were not isolated in one of the retail outlets in Baruwa, Ipaja, Lagos. For both samples (*P. senegalensis* and *M. poutasou*), *E. coli* was found to have occurred more than *Salmonella* spp. The presence of *E. coli* in smoked fish is in agreement with Adesoji *et al.* (2019) who reported that *E. coli* was the most prevalent bacteria isolated from smoked fish with an occurrence of 24.4%. The presence of *Salmonella* spp in smoked fish in this study is similar to the report of Balogun *et al.* (2019) who isolated *Salmonella* spp from Raniceps raninus (Tadpole fish). The variety of microorganisms found in these smoked fish could be as a result of exposure of the product at the market. The fish tissues have the ability to reabsorb water from the environment thereby creating a suitable condition for microorganisms to grow. The low sanitary conditions of the market and improper handling can also result in diversity of microorganisms found in smoked fish. It is noteworthy that *Staphylococcus aureus* is the most prevalent bacteria in the fish samples obtained from the four retail markets under this study. This is in agreement with the findings of Adebayo-Tayo *et al.* (2008) and Abolagba and Igbinevbo (2010) who reported the occurrence of *S. aureus* in smoked fish marketed in Uyo and Benin respectively.

CONCLUSION

In this study, the presence of bacterial pathogens shows the unhygienic status of the fish samples. It was necessary to determine the level of bacterial contamination of smoked fish in order to safeguard the public health of the consumers. Since smoked fish can harbor some food borne pathogens (*Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*), it is therefore important to exercise caution in consuming products that are displayed openly in order to prevent food borne diseases. Also, smoked fish should be sold in the markets under hygienic conditions to prevent the proliferation of bacterial pathogens.

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