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Antimicrobial analysis of isolates from sea food exposed to different preservation techniques

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Abstract

The study examined the microbial load of bacteria and fungi of samples stored from 0- 10 days. The study samples were subjected to microbiological analysis. The organisms isolated were characterized by using standard biochemical methods, the following bacterial were isolated *Salmonella* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Klebsiella* sp, *Serratia* sp, *Escherichia coli*, *Citrobacter* sp, *Enterococcus* sp, *Enterobacter* sp and *Proteus* sp. The study showed that *Staphylococcus* sp was more prevalent with a percentage occurrence of 19.0%. This was followed by *Pseudomonas* sp and *Klebsiella* sp with prevalence of 14.3% respectively. *Escherichia coli* and *Bacillus* sp were at a prevalence of 11.1% respectively. *Salmonella* sp occurred at 7.9% and *Enterococcus* and *Proteus* were 6.3% respectively while *Serratia* sp was the least occurring bacteria, also the percentage of occurrence of *Penicillium* sp stood at 77.8% while *Aspergillus* sp was least occurring with a percentage frequency of 22.2% respectively. Thus, reduction in water activity as a result of smoking fish slow down spoilage and extend shelf life of the sample. In the samples treated, smoking have been shown to be more effective in the preservation than Refrigeration.

Keywords: Antimicrobial; Spoilage; Microorganisms; Facultative

1. Introduction

Microbial spoilage is a common source of food spoilage, which occurs due to the action of microorganisms. It is also the most common cause of foodborne diseases. Perishable foods are often attacked by different microorganisms. The growth of most microorganisms can be prevented or lingered by adjusting storage temperature, reducing water activity, lowering pH, using preservatives, and using proper packaging (Tianji *et al.*, 2014). Microorganisms involved in food spoilage can be divided into three major categories, which are molds, yeasts, and bacteria. Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include prokaryotes (bacteria), single-celled organisms lacking defined nuclei and other organelles, and eukaryotes, single-celled (yeasts) and multicellular (molds) organisms with nuclei and other organelles. Some microbes are commonly found in many types of spoiled foods while others are more selective in the foods they consume; multiple species are often identified in a single spoiled food item but there may be one species (a specific spoilage organism, SSO) primarily responsible for production of the compounds causing of odors and flavors. Within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes, such as lactic acid bacteria and molds, secrete compounds that inhibit competitors (Gram *et al.*, 2002). Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly

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the bacteria, have changed in recent years and some older names are no longer in use. The Aim is to determine the effect of Antimicrobial Analysis of Isolates From Sea Food Exposed to Different Preservation Techniques. Yeasts are a subset of a large group of organisms called fungi that also includes molds and mushrooms. They are generally single-celled organisms that are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites. Yeasts can grow with or without oxygen (facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Spoilage molds can be categorized into four main groups: *Zygomycetes* are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. Generally they require high water activities for growth and are notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are zygomycetes. Some zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals. The most common spoilage species are *Mucor* and *Rhizopus*. Zygomycetes are not known for producing mycotoxins but there are some reports of toxic compounds produced by a few species. *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen). Some spore-formers are thermophilic, preferring growth at high temperatures (as high as 55°C). Some anaerobic thermophiles produce hydrogen sulphide (*Desulfotomaculum*) and others produce hydrogen and carbon dioxide (*Thermoanaerobacterium*) during growth on canned/ hermetically sealed foods kept at high temperatures, for example, soups sold in vending machines. Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures (Boor & Fromn, 2006). Other bacteria are associated with spoilage of chilled, high protein foods such as meat, fish, and dairy products. They may not be the predominant spoilage organisms but contribute to the breakdown of food components and may produce off-odors. Most species are aerobic although some grow at low oxygen levels and may survive vacuum packaging and one (*Brochothrix*) is a facultative anaerobe. Some examples include: *Acinetobacter* and *Psychrobacter*, which are predominant bacteria on poultry carcasses on the processing line and have been isolated from a variety of spoiled meat and fish. *Acinetobacter* grows at a pH as low as 3.3 and has been detected in spoiled soft drinks. These two genera do not produce extracellular lipases, hydrogen sulfide, or trimethylamine (fishy odor) and so are considered to have a low spoilage potential.

2. Material and methods

The study was carried out in vendors within and around Choba and Rivers state University campus, in Port Harcourt, Rivers State. The choice of both location is associated with population of people including students and staff of host tertiary institutions located around these study areas.

2.1. Sample Collection

A total of 36 samples made up of 12 Fishes, 12 Prawns, and 12 Periwinkles were purchased at random from Choba market, Port Harcourt. The samples were purchased and divided into 2 equal halves and wrapped in a sterile nylon bag. The samples were transported to the Microbiology laboratory within minimum time.

2.2. Sample Preparation

Each half of the samples were kept inside the freezer while the other half was smoked and samples analyzed within 2 days intervals (i.e day 2,4,6,8 and 10).

The Periwinkle sample was washed with clean water to remove debris or sand. The periwinkles were then roasted in a pot at minimal temperature and time with constant steering on the pot. The periwinkle was then brought out of the shell and divided into portions.

2.3. Characterization and identification of isolates

Colonies of different bacteria species were then picked out using a sterile inoculating loop and sub cultured for purification by streaking on plate count agar and incubated at 30°C for 24h. Individual colonies were characterized on the basis of their colony morphology and microscopic examination, biochemical characteristics.

2.4. Gram Staining

This is a common technique used to differentiate between gram positive and gram negative bacteria based on their cell wall constituents. It involves making heat fixing a colony that has been smeared on a microscope slide then followed by an application of a primary stain (crystal violet) for 60 seconds and then rinse with water then to the smear a mordant (gram iodine) was added for 60 seconds then rinse with water followed by the application of a decolorizer (95% alcohol) after 30 seconds to remove the unbound dye immediately rinse with water and then finally the application of a counter stain (safranin) for 30 seconds then rinse with water, air dry slide and viewed under microscope at $\times 100$ magnification.

2.5. Biochemical test

2.5.1. Catalase test

This test shall be done to differentiate between bacteria that produce the enzyme catalase from non-catalase producing bacteria. The enzyme hydrolyses hydrogen peroxide (H_2O_2) by breaking it down into water and oxygen gas. A drop of distilled water was placed on a slide and then a colony was picked and emulsified with the water using a sterile wire loop and emulsified with the water. Then a drop of hydrogen peroxide is added. The production of bubbles is an indication that oxygen was given off which indicates a positive test.

2.5.2. Citrate utilization test

This test is used to determine if the organisms can utilize citrate as its sole source of carbon and energy. The citrate test uses a medium in which sodium citrate is the source of carbon and energy. In Simon's citrate agar, the pH indicator is bromothymol blue, which is green at neutral pH and becomes blue when the medium becomes alkaline. Slopes/slants of Simon's citrate agar are prepared in Bijiou bottles and the test organisms shall be inoculated by streaking the surface, stabbing and butt with a sterilized inoculating needle and was then incubated at $35^\circ C$ for 48 hours and observed for a bright blue color in the medium which indicates a positive result.

2.5.3. Methyl red (mr) and voges-proskauer (vp) test

The test is made up of two tests; methyl red and voges-proskauer test. The methyl red indicates the production of sufficient acidic product from the fermentation of glucose while the voges-proskauer test indicates the ability to produce acetylmethylcarbinol. Nutrient broth (10 ml) shall be inoculated with the test organism and incubated for 24 hours. After incubation, 5 ml of test culture was transferred aseptically to a clean test tube for the vp test, 3-4 drops of methyl-red was added to first test tube. A positive reaction is indicated by a distinct red colour showing the presence of acid. A yellow color indicates a negative result. For the voges-proskauer test, 0.6 ml of alpha-naphthol and 0.2 ml of 40% potassium hydroxide is added to the second test tube. The broth was allowed to stand for 15 minutes for colour development after thorough agitation. If acetoin is produced, there will be a red colour change. A yellow to brown colour indicates a negative result.

2.5.4. Indole production

This test is used to determine the ability of certain microorganisms to breakdown amino acid tryptophan in the medium into indole in the presence of enzyme tryptophanase. The test organism shall be inoculated into test tubes containing 10ml of sterile tryptophan broth and incubated for 24 hours and examine for a red color in the surface layer after Kovacs reagent is added which indicates a positive result and no color change indicates a negative result.

2.5.5. Oxidase test

This test is used to determine the presence of cytochrome oxidase. Oxidase reagent is turned purple by organisms containing cytochrome C as part of their respiratory chain. Filter paper was soaked with a few drops of oxidase reagent. A colony was picked using a sterile wire loop and smeared on the filter paper. A deep blue or purple color was observed after 10-30 seconds which indicates positive; no blue color indicated a negative result.

2.5.6. Motility test

The motility test is used to determine if the organism is motile or not motile by moving away from the line of inoculation. Using a sterile wire loop an isolate is picked, stabbed directly into the center of the test tubes containing the nutrient agar and incubated for 18-24 hours and $37^\circ C$. A diffuse growth away from the line of inoculation indicates a positive result; no diffused growth indicates a negative result.

2.5.7. Triple sugar iron agar

Triple sugar iron agar as prepared and dispensed into test tubes autoclaved, slanted and allowed to cool. It involves inoculating into sterile test tubes using an inoculating needle and streaking across the top of the slant and incubate at 35-37 °C. After which colour change observed indicating a positive result and gas production will be observed.

2.5.8. Sugar fermentation

The triple sugar iron agar contains three sugar; glucose, sucrose and lactose in the ratio of 1:10:10. If the bacteria ferment sucrose and or lactose all the agar in the tube will turn yellow but if only glucose was fermented, the agar will turn yellow from the acid produced. Inoculate the organism into the test tube by stabbing (with needle) the agar tube to the bottom with the inoculum and streak across the top of the slant and incubate at 35°C for 24-48 hours. A black color observed in the tubes indicates a positive result.

2.5.9. Antibigram Screening

This was carried with methods as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012).

2.5.10. Antibiotics Susceptibility Testing (Ast)

0.1ml volume of the standardized culture will be aseptically inoculated into a 20 ml molten Mueller Hinton agar and gently swirled to effect mixing. The media was poured aseptically into a sterile Petri dish and allowed to solidify. The plate was labelled appropriately. This was repeated for rest of the isolated culture. After solidifying, a sterile forceps was used to implant the commercial multi-antibiotics disc unto the surface of the media aseptically. This culture media was incubated at 37 °C for 24 hours. After the incubation period, Petri dishes was examined and the zone of inhibition across the various antibiotics was determined.

3. Results and discussion

The organisms isolated from the study includes *Salmonella sp*, *Pseudomonas sp*, *Staphylococcus sp*, *Klebsiella sp*, *Serratia sp*, *Escherichia coli*, *Citrobacter sp*, *Enterococcus sp*, *Enterobacter sp* and *Proteus sp*. The study showed that *Staphylococcus sp* was more prevalent with a percentage occurrence of 19.0%. This was followed by *Pseudomonas sp* and *Klebsiella sp* with prevalence of 14.3% respectively. *Escherichia coli* and *Bacillus sp* were at a prevalence of 11.1% respectively. *Salmonella sp* occurred at 7.9% and *Enterococcus* and *Proteus* were 6.3% respectively while *Serratia sp* was the least occurring bacteria as shown Fig1 below.

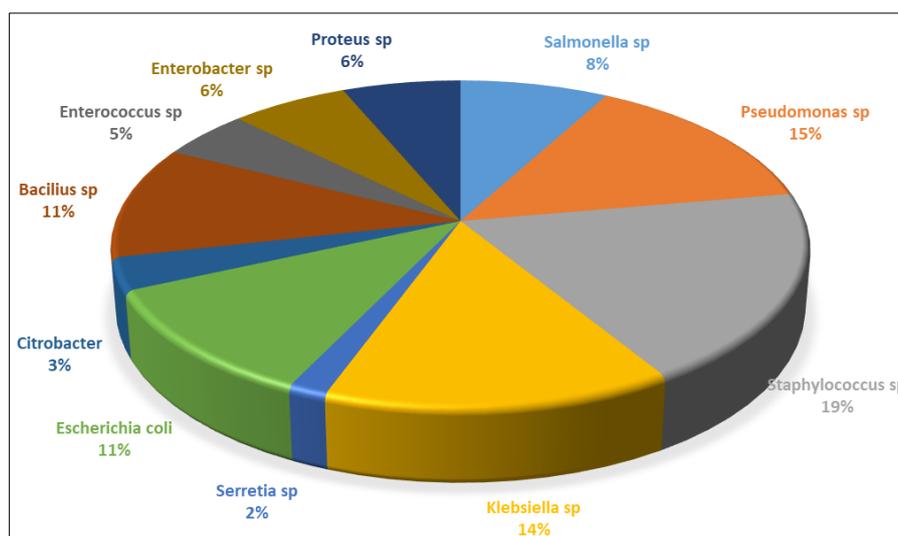


Figure 1 The Percentage frequency of bacteria isolated

The fungal isolated from the Fishes, Prawn and Periwinkles evaluated were *Aspergillus sp*. and *Penicillium sp*. The Percentage of Occurrence of *Penicillium sp* stood at 77.8% while *Aspergillus sp* was least occurring with a percentage frequency of 22.2% respectively. The result is shown in fig 2

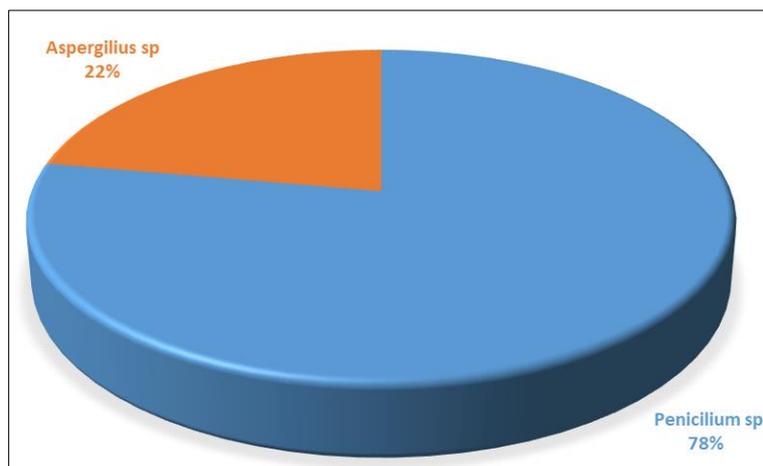


Figure 2 Pie chart of Percentage Frequency of occurrence of fungi isolated

The microbial evaluation of Fish, Prawn and Periwinkles stored by refrigeration and smoking was carried out in the study. A total of ten (10) bacterial genera were isolated from the study which include *Salmonella* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Klebsiella* sp, *Serratia* sp, *Escherichia coli*, *Citrobacter* sp, *Enterococcus* sp, *Enterobacter* sp and *Proteus* sp. The study showed that *Staphylococcus* sp was more prevalent with a percentage occurrence of 19.0%. This was followed by *Pseudomonas* sp and *Klebsiella* sp with prevalence of 14.3% respectively. *Escherichia coli* and *Bacillus* sp were at prevalence of 11.1% respectively. *Salmonella* sp occurred at 7.9% and *Enterococcus* and *Proteus* were 6.3% respectively while *Serratia* sp was the least occurring bacteria. Thus, reduction in water activity as a result of smoking fish slow down spoilage and extend shelf life of the sample. Consumption of Fishes, periwinkles and Prawns contaminated with these pathogenic bacteria species without subjecting it to a kill step such as smoking in order to reduce most of the microorganisms present in the periwinkles could have serious health implications. The bacteria flora of fresh molluscan shellfish which include periwinkle is largely dependent on the environment where they were harvested as well as handlers of the product and not the periwinkle. The Percentage of Occurrence of *Penicillium* sp stood at 77.8% while *Aspergillus* sp was least occurring with a percentage frequency of 22.2% respectively.

The possible sources of *Aspergillus* and *Penicillium* sp. in the periwinkle are water, soil and air where the spores of the fungi are commonly found. Various species belonging to the family *Nectiaceae* have useful commercial applications in some industries as biodegraders and biocontrol agents. In a related study, Ngozi *et al.* (2020) reported that *Aspergillus niger*, *A. flavus*, *Penicillium* sp. and *Mucor* sp. were present in dried periwinkle sold in a local market.

Escherichia coli is part of the intestinal flora of humans and vertebrates. The production of enterotoxins is associated with some strains of *Staphylococci* and *Bacillus* which poses a serious threat to consumers of food containing large population of these organisms (Ngozi *et al.*, 2020). *Pseudomonas aeruginosa* is a common opportunistic pathogen ubiquitous in nature. It is present in some blood infections, burns, and wounds. The symptoms range from mild diarrhea to profuse watery diarrhea which is the classical cholera.

4. Conclusion

The microbial evaluation of Fish, Periwinkles and Prawns sold within Choba preserved by smoking and freezing was carried and the result showed that ten bacteria and two fungi genera were isolated from the entire samples and there was a considerable reduction in number of viable cell count after some days of the storage methods used (Smoking and Freezing) although there was a considerable decrease in physicochemical and nutritional components of each of the stored samples. One of the major revolutionary inventions of human civilization was acquiring the knowledge to preserve foods as it was the precondition to man to settle down in one place and to develop a society. However, increasing shelf lives of food items without compromising original food properties is still critical and challenging. Food is an organic perishable substance, which is susceptible to spoilage due to microbial, chemical, or physical activities. Different traditional techniques, such as drying, chilling, freezing, and fermentation, had been evolved in the past to preserve foods and to maintain their nutrition value and texture. With time and growing demands, preservation techniques have been improved and modernized. To ensure food safety and long shelf life of foods, it is important to understand food spoilage mechanisms and food preservation techniques.

Compliance with ethical standards

Acknowledgment

John, G.N

Disclosure of conflict of interest

No conflict of interest to disclose.

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