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Public Health Implications of Microbial Contaminants in Seasoning Ingredients at Urban Slaughter Markets in Port Harcourt, Nigeria.

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ABSTRACT

This analysis explores the presence of microbial contaminants in seasoning ingredients sold in Port Harcourt's slaughter market, emphasizing the significant public health implications. Understanding the microbial landscape of these ingredients is crucial, as they are integral to food preparation and can influence the safety of meat products. The presence of harmful microorganisms in seasoning can lead to foodborne illnesses, affecting not only consumers but also the broader community's health. Identifying and mitigating these risks is necessary for promoting food hygiene, promoting public health, which protects vulnerable populations, particularly in areas with limited access to healthcare. Microorganisms play significant roles in food safety, particularly in seasonings, which are susceptible to contamination during production, sales and storage. This study investigates the microbial landscape of seasonings sold at the Slaughter Market in Port Harcourt, revealing a concerning prevalence of Total Heterotrophic Bacteria (THB) and Fungi (THF). Key findings indicate that certain samples, particularly Larsor Beef and Spicity seasonings, exhibit microbial counts exceeding acceptable limits set by food safety authorities. Lasor beef had THF counts of 3.1×10^2 CFU/g and THB count of 6.0×10^4 CFU/g; Kitchen Glory had THF counts of 2.1×10^2 CFU/g and THB count of 1.0×10^4; Spicity had THF count of 3.0×10^2 CFU/g and THB count of 1.7×10^4 CFU/g; Ludo Chicken had THF count of 2.1×10^2 CFU/g and THB count 5.0×10^4 CFU/g and Ongo stew had THF count of 5.0×10^2 CFU/g and THB count of 4.0×10^4 CFU/g. Biochemical analyses identified pathogenic bacteria such as *Salmonella* sp*, Staphylococcus* sp, *Aeromonas* sp. and *Acinobacter* sp. along with toxic and non-toxigenic genera of fungi such as *Aspergillus* sp*, Saccharomyces* sp*, Neurospora* sp*, Mucor* sp*,* and *Penicillium*. These microorganisms pose risks of food-borne illnesses and mycotoxin exposure, underscoring the urgent need for improved sanitation practices. The study underscores the importance of proper handling and storage to mitigate health risks associated with contaminated seasonings. Recommendations include strict quality control by manufacturers, consumer education on safe storage, and ongoing research into microbial threats in culinary ingredients. Addressing these issues will enhance food safety and protect public health, ensuring that seasonings enhance culinary experiences without compromising safety.

INTRODUCTION

Microorganisms are known to inhabit and thrive in food seasonings. This could be as a result of contamination before or after packaging. Seasonings are ingredients used for cooking. They can be used to add flavour or act as preservatives for food. They are used widely in all cultures and geographical locations. Seasonings are mostly used in food preparation towards the final stage the cooking (Ugboma *et al*., 2021; Silva and Ribeiro, 2024). Acids (such lemon juice), salt, and pepper are the most often used seasonings. When utilized correctly, seasonings enhance the flavors of the original foods without being tasted.

When seasonings are used properly, they cannot be tasted; their job is to heighten the flavours of the original ingredients. Seasonings have different components that act as source of nutrient for humans and microorganisms such as Fungi and Bacteria. Some of these components include; Spices (including cilantro), salt, dehydrated garlic and onion, and silicon dioxide added to prevent caking (Thomas *et al.,* 2021).

Despite being found in trace amounts in food, seasonings are known to be significant sources of microbial contamination because to the environmental circumstances surrounding their cultivation, harvesting, and processing. Additionally, foods containing seasonings are more likely to deteriorate and may have negative consequences due to potential neglects during sanitation or processing, keeping in mind the health hazards linked with mycotoxins produced by certain fungal species. (Koci-Tanackov *et al*., 2007). The kinds of bacteria and fungi present, the food's ecological state with regard to microorganisms, and the handling and storage circumstances all affect the likelihood of spoiling and the creation of mycotoxin. For instance, if the storage temperature is appropriate for the growth of bacteria and fungi, dried foods are prone to spoiling and the creation of toxins (Disegha, and Nmom, 2025). Additionally, they are kept in environments that encourage contamination by rodents, insects, and other rodents. Some of the spices available on the market have very low mycological quality, containing a wide variety of bacterial and fungal genera and species. Aerobic spore-forming bacteria

typically dominate the microbial ecology on a variety of spices and related materials. It was discovered that ginger, black and white pepper, celery seed, and paprika typically display total plate counts in millions per gram. (Farid. and Nareen, 2013). A growing number of signs suggest that eating food or utilizing raw materials tainted with fungi or mycotoxins can cause primary liver cancer and other severe illnesses. Heat resistance and the capacity to accumulate within the body were demonstrated by aflatoxins, ochratoxin, and sterigmatocystin.

(Hashem and Alamri, 2010).). Other mold species, such as *Penicillium, Scopulariopsis*, and *Sporendonema*, are commonly isolated from spices (Pickova *et al*., 2020). Seasonings can add flavour to our food and make it more enjoyable to eat. They can also stimulate our tastebuds and increase our appetite. Additionally, some seasonings like herbs and spices have antioxidant properties that may have health benefits.

Seasonings may not have any significant disadvantages when used in moderation. However, if used excessively, or if a person is sensitive to certain seasonings, it could potentially cause digestive issues or allergic reactions (Yashin *et al.,* 2017).

While most microorganisms in seasonings are harmless, improper storage or contamination can lead to the growth of harmful bacteria or molds. It is important to handle and store seasonings properly to prevent the risk of food-borne illnesses (Yashin *et al.*, 2017).

Seasonings are an integral part of culinary practices worldwide, enhancing the flavors and aromas of various dishes. They often consist of a diverse array of ingredients, including herbs, spices, salts, and other flavor enhancers. While these seasoning blends are designed to elevate the taste of our food, they can also harbor a hidden world of microorganisms that may pose health risks to consumers. The isolation and identification of these microorganisms are essential steps in understanding the potential risks and ensuring food safety in the culinary realm.

Both bacteria and fungi can be considered microorganisms in spices. Seasonings have been found to be contaminated by bacteria such Salmonella typhi, Escherichia coli, Staphylococcus aureus, Clostridium botulinum, and Listeria monocytogenes, which raises concerns about food-borne diseases and outbreaks (Dougkas et al., 2019). Fungi, represented by genera such as Asper*gillus, Penicillium, Fusarium*, and *Alternaria*, can also find a niche in seasonings. Some species within these genera are known for their ability to produce mycotoxins, posing additional health hazards (Deligeorgakis *et al.,* 2023).

This research aims to explore the microbial landscape of seasonings through the isolation and identification of these microorganisms. Understanding their presence and characteristics is pivotal for the formulation of effective food safety protocols. Additionally, it is essential for ensuring that seasoning products meet the standards for quality and safety, as microorganisms in seasonings can result in food-borne infections that cause symptoms like cramping in the stomach, diarrhea, and nausea.

MATERIAL AND METHOD

Material and reagents used, pipette, test tubes, rack, Sabouraud Dextrose Agar (SDA), cotton wool, wire-loop, bent rod, incubator, autoclave, filter paper, measuring cylinder, weighing balance, Bunsen burner, aluminium foil, hydrogen peroxide, Kovac's reagent, crystal violet, iodine, safranin, ethanol, Baritt reagent, methyl red, hot-air oven, etc.

Preparation of media

Two media were used for the research, they are; Sabouraud Dextrose Agar (SDA) and Nutrient Agar (NA). Both were prepared according to the manufacturer's specification, twenty-eight gram (28g) in 1000ml of distilled water for Nutrient Agar and 52g-1000ml for Sabouraud Agar dissolved in a conical flask was covered using cotton wool and aluminium foil, then autoclaved at 121^0C for 15 minutes.

When the medium was cooled to 45^0C, 20ml of the medium was poured into different petri-dishes and allowed to set.

Enumeration of Bacteria and Fungi

Serial dilution procedure was employed for the cultivation, identification and enumeration of Bacteria land fungal isolates from the samples. A ten-fold serial dilution technique was used to obtain appropriate dilution of the samples.

The surface of the dry, sterile Nutrient Agar (NA) and Sabouraud dextrose Agar (SDA) were plated with triplicate aliquots of the necessary dilution. While the SDA were kept at room temperature for three to five days, the infected plates on nutritional agar were incubated at 37^0C for twenty-four hours. Total Heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF) populations were determined by counting the number of ensuring colonies on the NA and SDA plates following the incubation time.

Isolation of Fungi

Viable colonies of fungi grew on Sabouraud Dextrose Agar (SDA) plates in the enumeration of population. Counts were gently transferred separately into freshly prepared SDA plates and incubated at ambient temperature for 3-5days. After incubation, the fungal isolates were investigated through microscopic and macroscopic examination.

Isolation and Identification of Bacteria

Discrete bacterial colonies developed on Nutrient Agar plates were randomly picked and sub-cultured unto a freshly prepared nutrient medium and incubated at 37^{o}C for 24hours.
The incubated bacteria isolates were later subjected to microscopic examination and biochemical tests. The tests include; starch hydrolysis, catalase test, citrate test, motility test, MRVP, indole, urease, sugar fermentation.

Maintenance of pure culture

All the isolates were stored in nutrient broth. The nutrient broth suspension was prepared by sterilizing in the autoclave at 121^0c for 15minutes. After sterilization and cooling of the stock bottle, the isolate growth was aseptically scraped from a lawn of heavily inoculated nutrient agar plate which had been incubated at 37^0c for 24hours.
A heavy suspension of the growth of each culture was prepared in 3ml bijon bottles. The suspension was thoroughly shaken and stored at 35^0c. The suspension broth serves as a long-term storage and preservation of isolate as well as a source of inoculation for weekly working culture.

Nutrient Agar and Sabouraud Dextrose Agar (SDA) used

Nutrient Agar (NA)

This is a general-purpose media that supports the growth of many microorganisms by providing them with organic growth factors.
The media was used for the estimation of total heterotrophic bacteria in the season spice samples. The preparation was done by weighing out 28g of the powder into 1000ml of distilled water and autoclave at 121^{o}C for 15 minutes.

Sabouraud Dextrose Agar (SDA)

It is a standard media used for the enumeration of fungi. The preparation was done by weighing 65g of the powder into 1000ml of distilled water according to the prescription of the manufacturer and sterilized at 121^{o}C for 15 minutes.
Samples of seasonings used in the research were all collected from Slaughter Market in Port Harcourt. They were Ludo Beaf Seasoning (LBS). Kitchen Glory Seasoning (KGS), Spicity Seasoning (SS), Larsor Chicken Season (LCS) and Onga Stew Seasoning (OSS).

Biochemical Tests

The following biochemical assays and Gram reactions were carried out: sugar fermentation, urease, catalase, oxidase, motility, indole, and Gram staining. Isolates were applied on a sterile slide, allowed to air dry, and then heated to fix them in order to perform the Gram staining process. After 30 seconds of crystal violet staining, the smear was rinsed and subjected to another 30 seconds of Lugol's iodine treatment before being decolorized. After 30 to 60 seconds of counter-staining with safranin, it was rinsed once again, allowed to air dry, and then examined under a microscope at 100x magnification using an oil immersion lens.

A bacterial smear was applied to filter paper that had been treated with oxidase reagent in order to perform the oxidase test. A rich purple hue after ten seconds of observation predicted a positive outcome, while no color change suggested a negative one. Half-strength nutrient agar was made in test tubes for the motility test. A straight wire pin was used to inoculate the organism, which was then cultured for 24 to 48 hours at 37°C. Motile bacteria grew diffusely throughout the media, but non-motile bacteria showed a distinct stab line. A little bacterial colony was put on a dry glass slide, and a drop of hydrogen peroxide was added as part of the catalase test. A successful outcome was demonstrated by the formation of oxygen bubbles.

In the urease test, urease activity was measured using urea broth that included phenol red. The effective hydrolysis of urea was signaled by a color shift from yellow to pink. The indole test involved inoculating a peptone water broth and incubating it for 24 to 48 hours at 37°C. A positive result was confirmed when 0.5 ml of Kovac's reagent was added, producing a pink ring.

Finally, using phenol red as a pH indicator, the sugar fermentation test assessed the formation of gas and acid in glucose, lactose, maltose, mannitol, and glycerol. A Durham tube was used to detect gas, and a color shift from red to yellow indicated the creation of acid. As ag (acid and gas), a (acid), g (gas), or N (neutral), the results were noted.

RESULTS

Results of microbiological analysis of the spices used for the research are presented in tables. Table 1 summarizes the microbial contamination levels in various seasoning samples, measured in Colony Forming Units per gram (CFU/g). Larsor Beef seasoning had the highest bacterial count at 6 x 10^4 CFU/g, indicating significant contamination, while its fungal count was 3 x 10^2 CFU/g. In contrast, Kitchen Glory seasoning displayed the lowest bacterial count at 1 x 10^4 CFU/g and a fungal count of 2 x10^2 CFU/g.

Spicity seasoning recorded a bacterial count of 1.7 x 10^4 CFU/g and a fungal count of 3 x 10^2 CFU/g. Ludo Chicken seasoning also had a high bacterial count of 5 x 10^4 CFU/g, with a fungal count of 2 x 10^2 CFU/g. Onga Stew seasoning showed the highest fungal count at 5 x 10^2 CFU/g, along with a bacterial count of 4 x 10^4 CFU/g. Larsor Beef had the highest bacterial

contamination, while Kitchen Glory had the lowest. Onga Stew exhibited the highest fungal contamination.

Table 2 presents the biochemical and sugar fermentation results for various isolates from seasoning samples, highlighting their characteristics and suspected organisms.

In the biochemical tests, several isolates demonstrated distinct patterns. For instance, Isolate 1 and Isolate 2 were identified as *Staphylococcus* sp. and *Salmonella* sp., respectively, both showing positive catalase activity (+) and varying results for sugar fermentation. Isolate 1 was positive for glucose, mannitol, lactose, and maltose, while Isolate 3 was positive for glucose and mannitol only.

Isolate 2, identified as *Aeromonas* sp., exhibited a positive oxidase reaction and showed fermentation of glucose, mannitol, and lactose. Isolate 5, identified as *Pseudomonas* sp., demonstrated positive reactions for catalase, oxidase, and both starch hydrolysis and urease tests.

Other isolates, such as Isolate 6 was identified as *Staphylococcus*, while isolate 9 was identified as *Salmonella* sp. Isolate 4 also indicated *Staphylococcus* with positive results for glucose and lactose fermentation, and Isolate 10 was identified as *Acinobacter* species.

In generals, the table highlights diverse microbial profiles across the samples, emphasizing the presence of pathogenic organisms, including *Salmonella* and *Pseudomonas*, and their corresponding biochemical characteristics, which are critical for assessing food safety.

Table 1: Macroscopic count of the samples

Sample Name	THF/CFU/g	THB/CFU/g
Larsor beef	$3.1x10^2$	$6.0x10^4$
Kitchen Glory	$2.1x10^2$	$1.0x10^4$
Spicity	$3.0x10^2$	$1.7 x10^4$
Ludo Chicken	$2.1x10^2$	$5.0 x10^4$
Onga Stew	$5.0x10^2$	$4.0 x10^4$

Key: THF = Total heterotrophic fungi; THB = Total heterotrophic bacteria.

Table 2: Biochemical and Sugar Fermentation results

Isolate	MMP	GLU	MAN	LAC	MAL	CAT	OXI	SH	CIT	ST	URS	VP	MR	IND	MOT	SO
1	GPC	A	A	A	A	+	-	-	+	+	-	+	-	-	-	*Staphylococcus* sp
2	GNR	Ag	A	Ag	A	+	+	-	-	-	-	-	+	-	+	*Aeromonas* sp
3	GPC	A	A	N	A	+	-	-	-	-	-	-	+	+	+	*Salmonella* sp
4	GNR	A	A	A	A	+	-	-	-	-	-	+	-	-	-	*Staphylococcus*
5	GPC	A	A	N	N	+	+	+	+	-	+	-	-	-	-	*Pseudomonas* sp
6	GNR	A	A	A	A	+	-	-	+	+	-	+	-	-	-	*Staphylococcus*
7	GNR	Ag	A	Ag	A	+	-	-	+	-	-	+	-	-	+	*Enterobacter*
8	GNR	N	A	A	N	+	-	-	-	-	-	-	+	-	-	*Flavobacter* sp
9	GNR	A	A	N	A	+	-	-	-	-	-	-	+	+	+	*Salmonella* sp
10OSS2	GNC	A	A	A	N	+	-	-	-	+	-	-	-	-	-	*Acinobacter* sp

Keys; MMR- Microscopic morphology, GLU- Glucose, MAN-Manitol, LAC-Lactose, MAL-Maltose, CAT-Catalase, OXI-Oxidase, ST-Salt Tolerance, CIT-Citrate, SH-Starch Hydrolysis, URS-Urease, VP-Voges Proskauer, MR-Methyl Red, IND-Indole, MOT-Motility, SO-Suspected Organism.

Table 3 provides macroscopic and microscopic descriptions of fungal isolates found in various seasoning samples, highlighting the characteristics of each suspected organism.

Mucor sp. was isolated from Larsor Beef seasoning, displaying a white fluffy growth with a white reverse coloration. Microscopically, it featured non-septate hyphae and mon-septate sporangiophores.

Rhizopus sp., found in Onga Stew and Ludo Chicken seasonings, exhibited a white cotton-like growth with grayish to blackish spots. Under the microscope, it also showed non-septate branched hyphae.

Aspergillus sp., identified in Kitchen Glory seasoning, presented a green lawn-like growth surrounded by a white halo. Microscopic examination revealed septate hyphae with non-septate round conidiophores.

Saccharomyces sp. was observed in Ludo Chicken seasoning, characterized by cream-colored, round glittering colonies and smooth oval-shaped budding cells.

Neurospora sp. was isolated from Spicity seasoning, noted for its yellow coloration.

Penicillium, found in Onga Stew and Ludo Chicken seasonings, exhibited green pigmentation with a white powdery surface and elevated center. Microscopic analysis showed erect, branched septate conidiophores.

Table 3: The Macroscopic and Microscopic description of fungal isolates found in the seasoning samples Reorganize table so that seasons will come after suspected organism

Sample name	Suspected organism	Macroscopic	Microscopic
Spicity Ludo Chicken Lasor Beef	*Mucor sp.*	White fluffy growth with reverse white colour.	Non-septate hyphae with mon-septate sporangiophores.
Ludo Chicken Kitchen Glory Seasoning Onga Stew	*Rhizopus sp.*	White cotton growth with grayish to blackish spots.	Non-septate branched.
Kitchen Glory	*Aspergillus sp.*	Green lawn like growth surrounded by white hallow with reverse white colour.	Septate hyphae with non-septate round conidiophores.
Onga Stew Seasoning Ludo Chicken Seasoning	*Saccharomyces sp.*	Cream coloured round glittering colonies.	Smooth oval shaped budding cells.
Kitchen Glory Seasoning Spicity Seasoning	*Neurospora sp.*	Yellow colour.	
Ludo Chicken Seasoning Onga Stew Seasoning	*Penicillium*	Green pigmentation with white background powdery surface with elevated centre.	Conidiophores septate erected and branched.

DISCUSSION

The results indicate that Total Heterotrophic Bacteria (THB) counts were highest in the Larsor Beef followed by Ludo Chicken and Onga Stew in decreasing order, while lower counts were observed in others such as Kitchen Glory and Spicity. Notably, Larsor Beef seasoning exhibited the highest bacterial count at 6.0 x 10^4 CFU/g, alongside a fungal count of 3.1 x 10^2 CFU/g. Kitchen Glory seasoning had the lowest bacterial count at 1.0 x 10^4 CFU/g and a fungal count of 2.1 x 10^2 CFU/g. Onga Stew seasoning showed the highest fungal count at 5.0 x 10^2 CFU/g. These findings underscore the potential for microbial contamination in seasonings, which could pose risks to consumer health (Al-Mazrouei *et al.,* 2024).

These findings align with previous research that identified high microbial loads in processed foods, particularly spices and seasonings (Xiaoyang, 2025). A study analyzing microbial contamination in 41 common food spices highlighted the prevalence of various pathogenic and spoilage microorganisms (Jaffee *et al.*, 2018). The importance of the current findings is further supported by research that indicates poor handling and storage conditions can result in higher levels of bacterial and fungal contamination. Bacillus species, Clostridium perfringens, Listeria monocytogenes, different Salmonella serotypes, Pseudomonas species, pathogenic Escherichia coli, and Staphylococcus aureus, for instance, can all contaminate spices (Xiaoyang, 2025).

Significant issues with food safety and public health are brought up by the high levels of microbial contamination, particularly in Larsor Beef and Onga Stew (Al-Mazrouei et al., 2024).

The presence of pathogens such as *Salmonella* and *Pseudomonas* signifies potential health risks (Al-Mazrouei *et al.,* 2024). These findings suggest that consumers may be at risk of foodborne illnesses, highlighting the need for stricter quality control measures in the production, processing and storage of seasonings (Tang, 2025).

The study's limitations include a small sample size and the lack of comprehensive testing for all potential pathogens. Additionally, the samples were collected from a single location, which may not represent wider trends in microbial contamination. The study also did not account for factors such as

environmental conditions, storage practices, and the source of raw material, which could significantly influence microbial counts (Tropea, 2022).

The presence of pathogenic organisms such as *Salmonella* and *Pseudomonas* signifies potential public health risks. Understanding the microbial composition of these seasonings is crucial for assessing their safety and ensuring consumer protection. Spices, even with their antimicrobial properties, can still harbor pathogens, as demonstrated by the presence of *Salmonella* in dehydrated garlic and fresh onions in past outbreaks (Al-Mazrouei *et al.,* 2024).

Future studies should focus on a larger variety of seasoning samples and include a broader range of pathogens. Examining how various storage conditions affect microbial development may yield important information on reducing contamination. Furthermore, it is necessary to investigate the efficacy of different intervention techniques, including irradiation, steam treatment, and cutting-edge non-thermal technologies like UVC-LED and cold plasma (TSulieman et al., 2023).

CONCLUSION

This study reveals significant microbial contamination in seasoning samples, particularly in Larsor Beef and Onga Stew. The presence of pathogenic organisms highlights the urgent need for enhanced food safety measures. Future research should focus on understanding and mitigating these microbial risks. A risk-based approach, incorporating preventive controls, validated kill steps, and stringent sanitation protocols, is essential for regulatory compliance and consumer protection. Overall, the findings emphasize the importance of vigilant food safety practices, as even seemingly harmless seasonings can pose health risks.

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