



Microbiological Assessment of Smoked Pork Meat Sold in Oghara and Sapele Local Markets in Delta State, Nigeria

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Article Information

Article # 10296

Received: 19th Sept. 2025

1st Revision 25th Sept. 2025

2nd Revision: 7th Oct 2025

Acceptance 19th Oct 2025

Available online:

15th October 2025.

Keywords

Food-borne infections

Personal hygiene

Contaminations

Antibiotic tests

Pork meat

Abstract

In recent times, Nigerians have become more and more dependent on smoked pork as a result of a several factors, including the nation's rapid population growth, urbanisation, unemployment, and workplace pressures, and lifestyle changes that have made people more mobile and transient and largely reliant on these relatively inexpensive foods for nutrition. In Oghara and Sapele, smoked pork can be found sold in a range of public areas, such as parking lots, schools, markets, and roadside stands. Regarding the microbial contamination or hygienic preparation of these products, there is, however, no guarantee. This study aims at evaluate smoked pork sold in Oghara and Sapele, Delta State, for microbial contamination and antibiotic sensitivity of the isolated microbes. A total of sixty (60) pork samples were used in the study for over a period of 4 weeks. This comprises three (3) samples each collected from five locations in Oghara and Sapele– Amukpe Market, Amukpe Roundabout, Okirigwe Park, Oghara Junction and Ogharefe Market. The microbial concentrations were determined using a standard microbiological method. The results revealed the presence of *E. coli*, *Klebsiella* species, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella*. The antibiotics sensitivity test revealed that most of the isolated microorganisms were resistant to Ofloxacin, Azithromycin, Augmentin, and Nitrofurantoin. The results from this study indicated that cross-contamination from the hands of vendors to customers can happen frequently. The findings suggest that the pork that was sold in Oghara and Sapele, Delta State may contain pathogenic bacteria that may lead to onward transmission of foodborne infections, hence not be fit for human consumption and pose a risk to health.

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Introduction

Meat has been said to have an excellent protein profile because it contains all of the essential amino acids that the body needs (Orjiakor *et al.*, 2021). A sizable portion of the world's population eats meat as their main food source. Humans can contract enteric bacteria by consuming undercooked meat products (Ateba and Setona, 2011). The scientific evidence that meat contains more protein and vitamins (especially A and B12) than plant sources also lends credence to the nutritive significance of meat. About 5000 years ago, pigs were first domesticated (Pond *et al.*, 2005). Pigs have a great potential to meet the world's growing meat demand because of their high prolific rate, short gestation period, excellent climate and food adaptation, and high feed conversion ratio (Pond *et al.*, 2005). It has been shown that pork, which makes up about 40% of all meat produced, is a valuable food source worldwide. Pork is one of the most perishable of all the important foods because it contains enough nutrients to encourage the growth of microorganisms.

Though it can spoil easily and lead to foodborne illnesses in humans, meat is a great source of protein for a diet and can have detrimental effects on one's health and finances (Komba *et al.*, 2012). Even though healthy animals' muscles don't contain any microbes, meat tissues can get contaminated during the course of transportation and slaughter (Ercolini *et al.*, 2006). Potential sources of contamination include heavily contaminated utensils, benches used for handling pork, storage at the retailer's booth or store, and the abattoir. Pork contamination can result from a variety of causes, including improper handling and storage of finished pork products, bowel rupture during the evisceration process, and indirect contamination from tainted water. Although there are many different kinds of microbes present in fresh meat, they can become dominant depending on the raw meat's P^H, composition, texture storage temperature, and mode of transportation (Adu-Gyamfi *et al.*, 2012). Human health is at risk from raw meat because it can harbour a variety of dangerous bacteria, including *Salmonella*

species, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli*, *S. aureus*, and, to a lesser degree, *Listeria monocytogenes*. In order to avoid food-borne illnesses, these pathogens need to be appropriately handled and controlled (Norrung *et al.*, 2009).

The most likely sources of contamination are the animal's death, the process of eviscerating the meat, the water used to wash the meat, the handling of the butchers, the environment's pervasive microorganisms, the hygienic conditions of the spices used to flavor the pork, and the equipment used during the process, such as knives, skewers, and other utensils (Igyor and Uma, 2005). Given the rising popularity of pork, its susceptibility to bacterial growth and habitation, the perishable nature of meat, and the association between pork consumption and food poisoning which includes cases of documented gastroenteritis, or inflammation of the stomach and intestines after consuming pork there may be a risk to food safety, particularly if the food is prepared in an unhygienic way with a high risk of contamination and is ready to eat.

The demand for ready-to-cook and ready-to-eat meat products has increased as a result of the rapid urbanization and fast-paced lifestyle that have drastically changed societal eating habits. Nowadays, consumers are pickier about quality and concerned about the affordability, freshness, and health advantages of meat products. Meat processing, preservation, and slaughtering may cause a variety of biochemical changes in the meat as well as microbial contamination. In addition, it is easily spoiled and often associated with the transmission of food-borne illnesses (Olaoye and Nilude, 2010). Approximately 69% of gram-negative bacteria are known to be responsible for bacterial foodborne illnesses (Okonko *et al.*, 2010). The meat samples were contaminated with high concentrations of *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Proteus vulgaris*, *Staphylococcus aureus*, and *Bacillus* spp., according to several investigators' reports (Orjiakor *et al.*, 2021). However, food-borne pathogens can migrate from contaminated meat and attach themselves to surfaces, thereby spreading illnesses throughout the community. In Oghara, Delta State, Nigeria, smoked pork can be found sold in a range of public areas, such as parking lots, schools, markets, and roadside stands. Regarding the microbial contamination or hygienic preparation of these products, there is, however, no guarantee. The need for this investigation resulted from a lack of understanding about food safety protocols and the extent of microbial contamination in smoked pork.

This study aimed to assess smoked pork sold in Oghara and Sapele, Delta for microbial contaminations and antibiotic sensitivity of the isolated microbes. This is to gain a better understanding of the level of microbial contamination and the effect of antibiotic resistance.

Materials and Methods

Description and Selection of the Study Area

Oghara is a tropical city in Delta State's Ethiope West local government area. It is located in Nigeria's belt of tropical rainforests. Oghara, which is composed of the three subclans Ogharefe, Ovade, and Oghareki, is situated at latitude 5°45'47.24'E and longitude 5°59'0.83' N. It is ruled by a chieftaincy system, with the Ovie of Oghara serving as the monarch. During the wet season, the area experiences a relatively high temperature of 25°C to 27°C; however, during the dry season, the temperature rises slightly to 27°C to 32°C. Sapele is also a tropical city in Delta State's Sapele local government area. Although there are a variety of Nigerian ethnic groups represented in this metropolis, the Urhobo-speaking tribe of Delta State makes up the majority.

Collection of samples

The smoked pork samples were collected weekly over a period of 4 weeks. On each sampling occasion, the smoked pork samples were collected in accordance with standard protocol. (ISO 17605:2016) From five selected locations in Oghara and Sapele Delta State, Nigeria and a total of 60 samples were collected aseptically with sterile polythene bags and transported to the laboratory immediately for analysis.

Sample size

A total of sixty (60) pork specimens were used in the study. This comprises of three (3) collections, each from the five locations in Oghara and Sapele – Amukpe Market, Amukpe Roundabout, Okirigwe Park, Oghara Junction and Ogharefe Market.

Sample processing

The vended pork was subjected to microbiological techniques for the isolation, identification and antibiotic susceptibility testing in the laboratory.

Preparation of Culture Media

The media used were prepared according to the manufacturer's instructions. The media used were MacConkey agar, Mannitol Salt Agar, EMB, Blood Agar and Nutrient Agar.

Inoculation, Isolation, Characterisation and Identification

Inoculation

One gram from each food sample was aseptically transferred into sterile test tubes containing sterile

water, and 10-folds serial dilutions was done for plate counts. Then a sterile wire loop was used to take inoculum from the stock and streaked on the culture media accordingly. The plates were incubated at 37°C for overnight

Identification of isolates

Gram staining and other biochemical tests were carried out for the characterisation and identification of the organisms: Catalase, Coagulase, Citrate utilization, Urea decomposition, Indole, Sugar fermentation and Triple sugar iron (TSI) agar tests were used for the identification of isolates.

Gram Staining Reaction

Gram stain or Gram staining, also called Gram's Method, is a method of staining used to distinguish and classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria (Colco, 2005).

Principle: Gram reaction differentiates between gram-positive and gram-negative bacteria based on the differences in permeability of the cell wall to peptidoglycan during staining. Gram positive bacteria have more peptidoglycan, thereby retaining the crystal violet, whereas the gram-negative bacteria lose the primary stain and take the colour of the counterstain (Cheesbrough, 2004).

Procedure:

A thin smear of each isolate of the organism from the culture plate was made by emulsifying in a drop of normal saline on a clean grease-free glass slide air-dried, and heat fixed by gently passing through flame. The glass slide is then out on a staining rack and flooded with crystal violet for 60 seconds. It was then rinsed in water and treated with Lugol's iodine for 60 seconds, and rinsed with water. The smear was decolorized briefly with acetone, which was rinsed off immediately with water. The smear was counterstained with safranin for 60 seconds and rinsed with water. The back of the slide was cleaned, and the slide was left to dry. Thereafter, a drop of immersion oil was placed on the stained smear and it was examined under a microscope using x100 objective lens (oil immersion). Gram-positive bacteria will stain blue/purple, and Gram-negative bacteria will stain pink/red (Cheesbrough, 2006). *Escherichia coli* is used as gram negative control, while *Staphylococcus aureus* serves as Gram-positive control.

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase-producing bacteria such as streptococci.

Principle: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure:

Pour 2–3 ml of the hydrogen peroxide solution into a test tube. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. *Important:* Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur. Immediate bubbling indicates a positive result, while no bubbling indicates a negative result.

Coagulase test

This test is used to identify *S. aureus*, which produces the enzyme coagulase.

Principle: Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*: Free coagulase, which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test.

Bound coagulase (clumping factor), which converts fibrinogen directly to fibrin without requiring a coagulation factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

Procedure:

Place a drop of distilled water on each end of a slide or on two separate slides. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar. Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Indole test

Testing for indole production is important in the identification of enterobacteria. Most strains of *E. coli*, *Proteus vulgaris*, *Proteus rettgeri*, *Morganella morganii*, and *Providencia species* break down the amino acid tryptophan with the release of indole. Some organisms can split the amino acid tryptophan to

form the compound indole. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products – one of which is indole.

Principle: The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent, which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red-coloured compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Procedure:

The test isolates were inoculated into peptone water prepared by sterilizing at 121°C for 15 minutes and subsequently incubated at 37°C for 24 hours. 3 drops of Kovac's reagent was added and monitored for a few minutes. Formation of red rings indicates a positive test, while yellow ring shows a negative test (Cheesbrough, 2004).

Citrate utilization test

This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonium as its only source of nitrogen. The citrate is metabolized to acetoin and carbon dioxide.

Procedure:

Simmons citrate agar was prepared in a slant in bijou bottles and sterilized at 121°C for 15 minutes. After solidification of the agar, the medium was aseptically inoculated with the test isolate using a sterile wire loop. The agar was incubated at 37°C for 24-48 hours. After incubation hours the slants were examined for colour change. Positive reaction turns blue while organisms that are unable to utilize citrate remain green. (Cheesbrough, 2004).

Urease test

Testing for urease enzyme activity is important in differentiating enterobacteria. *Salmonellae* and *Shigellae* are urease-negative.

Principle: The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

The entire Christensen's urea agar slant was inoculated with the isolates and incubated at 37°C for 24 hours, and it was observed for colour change and this reaction turns the alkaline medium by changing the indicator to red-pink (Cheesbrough, 2006).

Triple Sugar Iron Agar Test (TSI)

Triple sugar iron agar (TSIA) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulphate, and the pH indicator phenol red. The test is used to differentiate enteric organisms based on the ability to reduce sulphur and ferment carbohydrates (Barrow and Feltram, 2004). An angled well-oxygenated area is called the slant at the top, and a poorly oxygenated area on the bottom is called the butt.

As with the phenol red fermentation broths, if an organism can ferment any of the three sugars present in the medium, the medium will turn yellow. If an organism can only ferment glucose (dextrose), the small amount of dextrose in the medium is used by the organism within the first ten hours of incubation. After that time, the reaction that produced acid reverts in the aerobic areas of the slant, and the medium in those areas turns red, indicating alkaline conditions (Barrow and Feltram, 2004). The anaerobic areas of the slant, such as the butt, will not revert to an alkaline state, and they will remain yellow. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate at the butt.

A wire loop of isolate culture was inoculated by stabbing into the Triple sugar iron gel and streaking over the surface of a slope of the agar which was incubated overnight at 37°C for 18-24 hours, and was examined for sugar fermentation (colour change at the butt and slant), gas production and hydrogen sulphide production.

Control: *Proteus mirabilis* produced gas H₂S, yellow butt and red slope while *Pseudomonas aeruginosa* (control) produced no gas, no H₂S but red colour at both butt and slant (Cheesbrough, 2006).

Sugar Reaction

A group of sugar tests comprises certain biochemical tests which are used in the identification of enterobacteriaceae.

The sugars used are D-maltose (C₆H₁₄O₆), D-glucose (C₆H₁₂O₆H₂O), Sucrose (C₆H₂₂O₁₁) and D-lactose (C₆H₂₂O₁₁).

Procedure:

With the aid of a sterilized wire loop, the colony was picked and inoculated into the different sugars (Lactose, Maltose, Sucrose and Glucose) and incubated at 37°C for 24 hours. The different colour changes were observed for each of the sugars (Cheesbrough, 2006).

All the results of the analyses were compared with a standard table for the identification of enterobacteria and other enteric organisms (Cheesbrough, 1994).

Detection of microorganisms associated with the smoked pork meat sold in Oghara and Sapele

The detection of *E.coli*, *Klebsiella* spp, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella* spp was determined using standard microbiological methods (ISO 6579:2005)

Antibiotic susceptibility testing of the isolates

The isolates were subjected to antibiotic sensitivity testing using the disc diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI, 2012).

Statistical Analysis

The data obtained from this study was analysed using Microsoft Excel 2016 version, and the results were presented in percentages, figures and tables.

Results

The results of the microbial evaluation of vended pork meat from selected vendors in Oghara and Sapele, Delta State, are presented in the following figure and tables.

Figure 1 shows the total heterotrophic bacteria count from 60 vended pork samples in this study from Amukpe Market, Amukpe Roundabout, Okirigwe Park, Oghara Junction and Ogharefe Market. Oghara Junction had the highest bacteria count in week one (1.58×10^6 CFU/g), while Oghara Junction in week three and Amukpe Roundabout in week four had the lowest bacteria count (6.9×10^6 CFU/g).

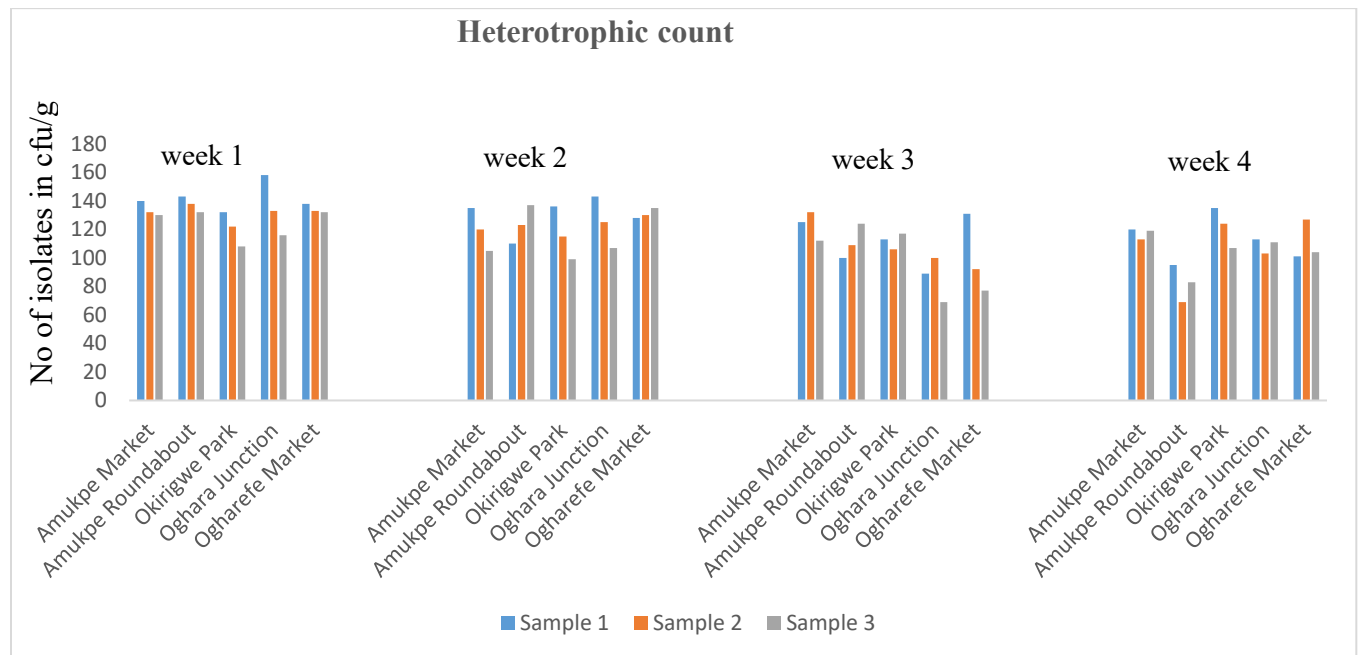


Figure 1: Total heterotrophic bacteria count

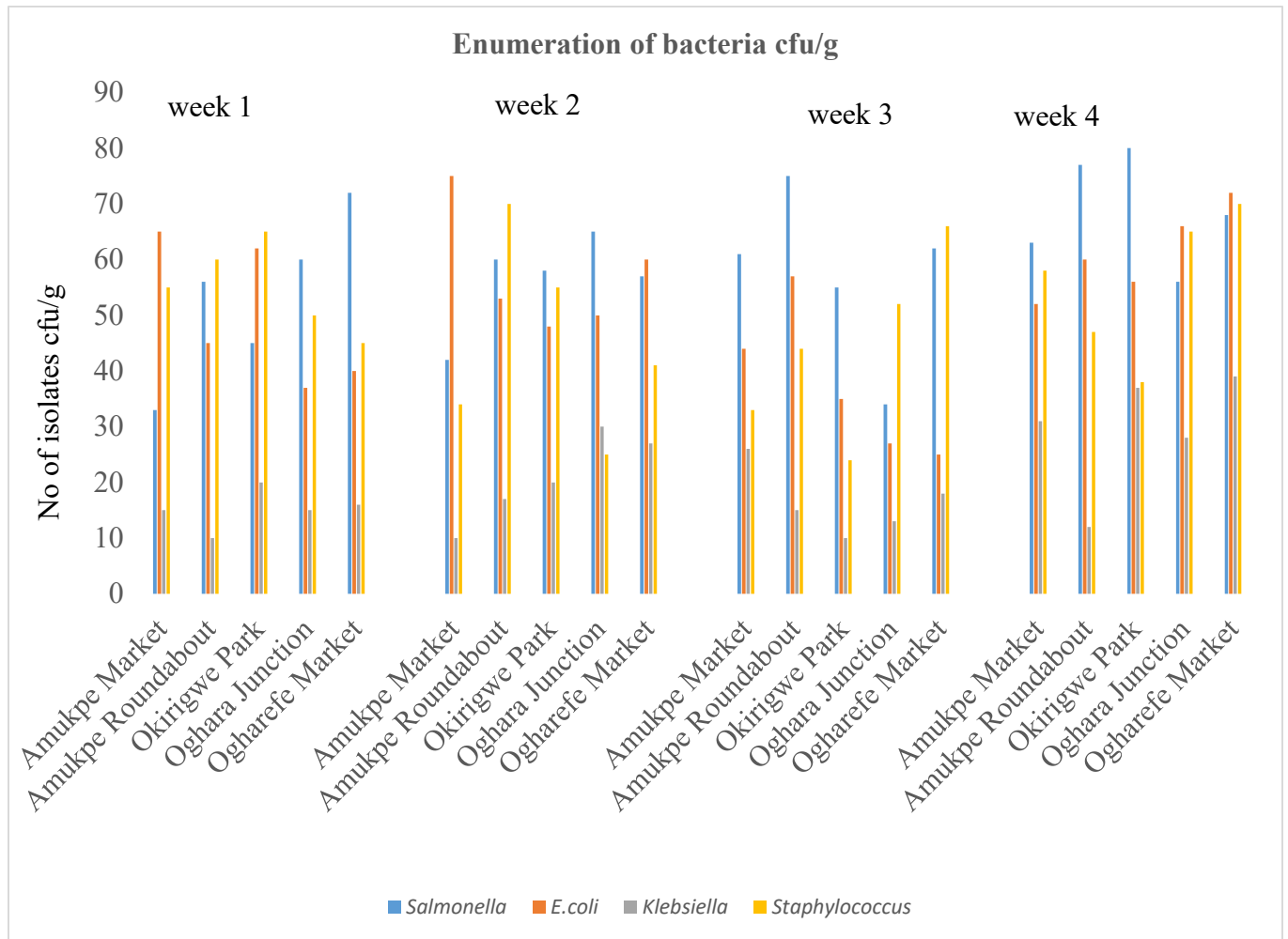


Figure 2: Enumeration of bacteria based on selective media in cfu/g

Table 1: Morphological and Biochemical Characteristics of Isolates

Colony	Colony Morphology	Gram Reaction	Biochemicals				Sugars				Suspected Organisms
			Citrate	Urease	Catalase	Indole	Glucose	Sucrose	Lactose	Maltose	
MCC _P	Large, circular, convex, smooth and opaque Rod, green metallic sheen with dark centre	-	-	-	+	+	+	+	+	+	<i>Escherichia coli</i>
EMB _P	Flat, rough, creamy Rods in clusters	-	+	+	+	-	+	+	+	+	<i>Klebsiella</i> spp.

MSA _P	Convex, rough, Golden yellow Cocci in clusters	+	+	+	+	-	+	+	+	+	<i>Staphylococcus aureus</i>
MSA _C	Convex, rough, creamy Cocci in single	+	-	+	+	-	+	+	+	+	<i>Staphylococcus epidermidis</i>
BA _w	Convex, rough, Golden yellow, Cocci in clusters, non-motile	+	-	+	+	-	+	+	+	+	<i>Staphylococcus aureus</i>
BA _C	Rod-shaped, motile, short chains	-	+	-	+	-	+	-	-	+	<i>Salmonella</i> spp

Keys: + = Positive ; - = Negative

Table 1 shows the results of the cultural, morphological, biochemical and sugar fermentation test of isolates obtained from the various samples. From the table below, five (5) microorganisms were identified, namely: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella* spp and *Salmonella*

Table 2: Antibiotics Sensitivity Test

Gram-positive Organisms										
Isolates	OFL	AZM	CXM	CIP	GEN	LVX	PEF	CRO	RI	
Mm										
<i>Staphylococcus aureus</i>	0 (R)	0 (R)	27.5(S)	15 (I)	25(S)	15 (I)	20 (S)	0 (R)	0.375	
<i>Staphylococcus epidermidis</i>	0 (R)	8 (R)	15 (I)	10 (R)	8 (R)	15 (I)	15 (I)	0 (R)	0.625	
Gram-negative Organisms										
Mm										
	OFL	CAZ	AUG	CIP	GEN	LVX	NIT	CRO	RI	
<i>Escherichia coli</i>	24 (S)	0 (R)	0 (R)	27 (S)	23 (S)	25 (S)	7 (R)	0 (R)	0.5	
<i>Klebsiella</i> spp.	0 (R)	0 (R)	10 (R)	10 (R)	15 (I)	0 (R)	0 (R)	0 (R)	0.875	
<i>Salmonella</i> spp	20 (S)	0 (R)	23 (S)	8 (R)	15 (I)	15 (I)	0 (R)	0 (R)	0 (R)	

Keys: RI = Resistance index: Resistance (R) = 0 – 10 mm: Intermediate (I) = 11 – 19 mm: Susceptible (S) = > 20mm

Discussion

This result implies that unhygienic pork handling in the street food stall can increase its microbial contamination, which is supported by findings from DangXuan *et al.* (2019), who evaluated cross-contamination during pork preparation. The vended pork evaluated in this study was contaminated with different bacteria. Similar to the study carried out by Orjiakor *et al.* (2021) in Oghara on fresh and smoked pork, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis*, which were reported in this study, were also isolated and identified from the sampled pork in their study. However, *Salmonella* spp. were also detected, which is in tandem with the report by Ngo *et*

al. (2021) in Vietnam. This risk is especially high for food handlers who may lack the awareness and skill to ensure food safety (Tran *et al.*, 2018). The result indicated that *Escherichia coli*, *Klebsiella* and *Salmonella* spp., were the only gram-negative bacteria isolated and identified in this study, while *Staphylococcus aureus* and *Staphylococcus epidermidis* were the gram-positive bacteria reported in this study, which is in line with the report of Iche and Anyanwu (2022). Interestingly, in a study carried out on fresh and smoked pork in Oghara, similar to this study, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis* were the bacteria isolated and identified (Orjiakor *et al.*, 2021).

Escherichia coli, *Klebsiella* and *Salmonella* spp. were reported in samples from all locations, while *Staphylococcus epidermidis* was present in samples from all locations except Amukpe Roundabout and *Staphylococcus aureus* was only detected in samples from Amukpe Roundabout. This is similar to the reports of Thanigaivel and Anandhan (2015), Awe and Adejo (2017), Karoki *et al.* (2018), Koirala *et al.* (2021), Orjiakor *et al.* (2021), and Wata *et al.* (2024), where *E. coli* was reported, indicating their wide distribution across different geopolitical zones, climate and food products.

Results from the antibiotics susceptibility test indicated that *Staphylococcus aureus* was observed to be resistant to Ofloxacin, Azithromycin and Ceftriaxone but susceptible to Gentamycin, Pefloxacin and Cefuroxime. This is similar to the report of Wata *et al.* (2024) where *S. aureus* was susceptible to Gentamycin, but resistant to Pefloxacin. *Staphylococcus epidermidis* showed resistance to Ofloxacin, Azithromycin, Ceftriaxone, gentamycin and Ciprofloxacin. However, in the report by Orjiakor *et al.* (2021), 99% of the isolates were susceptible to Ciprofloxacin. *Escherichia coli* was susceptible to Ofloxacin, Levofloxacin, Gentamycin and Ciprofloxacin but resistant to Ceftazidime, Augmentin, Nitrofurantoin and Ceftriaxone while *Klebsiella* spp., showed resistance to all antibiotics used except Gentamycin. This is in contrast with the report of Wata *et al.* (2024) where *E. coli* was resistant to Gentamycin and *Salmonella* species were only susceptible to Ofloxacin and Augmentin.

The widespread distribution of the meat product makes the consequence of contamination with food poisoning microorganisms more serious. The isolation of these organisms from roasted pork is of public health importance because they may be pathogenic organisms and it is worrisome, because many people like to consume this food product, especially in Oghara and Sapele Delta State. *Staphylococcus aureus* produces a variety of extracellular enzymes and toxins that are responsible for food poisoning and can rapidly develop resistance to many antimicrobial agents and pose a therapeutic problem. The presence of these microbes in vended foods suggests improper hygiene in food handling and processing, insufficient heating of food, or failure to adhere to the standard protocols of food handling by the staff working in the eateries (Oluboyo *et al.*, 2020).

Conclusion

An overview of the microbiological assessment of pork sold in Oghara and Sapele, Delta State, Nigeria, is given in this study. It also indicates that cross-

contamination from the hands of vendors to the pork sold to customers can happen frequently. The pork meat that was sold in Oghara and Sapele was found to be contaminated by *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella* species. These findings suggest that the pork that was sold in Oghara and Sapele may not be fit for human consumption in general. It is therefore recommended that preventing food contamination is the most efficient method of preventing food-borne infections. Cutlery and other food processing utensils, including pots, knives, and other utensils, as well as eating utensils and plates, need to be thoroughly cleaned and sterilized. In order to prevent cross-contamination, food handlers must also regularly change their hand gloves, wear hair nets, and wash their hands thoroughly. As much as possible, eating areas and counters should be sanitised to avoid cross-contamination and food-borne infections. In order to keep an eye on food vendors' operations and their adherence to food safety laws and regulations, the government should establish organisations and bodies dedicated to food safety. It is hereby advised to regularly carry out microbial evaluation on food samples, as this may provide additional insight into the infectious potential of the contaminated samples.

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