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Isolation and Identification of Pathogenic Bacteria Associated with Tomatoes, Bananas, Spinach and Okra commonly sold at Old Market, Patigi, Kwara State, Nigeria

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ABSTRACT: Fresh fruits and vegetables are vital components of a healthy diet but are often linked to foodborne illnesses due to microbial contamination. Thus, the objective of this paper is to isolate and identify pathogenic bacteria associated with Tomatoes (*Solanum lycopersicum*), bananas (*Musa spp*.), spinach (*Spinacia oleracea*), and okra (*Abelmoschus esculentus*) commonly sold at Old Market, Patigi, Kwarra State, Nigeria using standard microbiological techniques. Bacterial load of fresh produce range from 0.7 x 10^4 - 1.8 x 10^4 and from 3.3 x 10^4 -7.0 x 10^4 in spoiled produce.The total bacterial load was higher in spoiled produce, with spoiled bananas recording 7.0 × 10^4 cfu/ml, while fresh okra had the lowest bacterial load of 0.7 × 10^4 cfu/ml. Morphological and biochemical analyses identified *Escherichia coli*, *Salmonella spp.*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Enterobacter aerogenes*. *Klebsiella pneumoniae* was the most prevalent species, occurring in 66.67% of banana samples, 33.33% of spinach samples, and 33.33% of tomato samples. These findings highlight significant microbial contamination of fresh and spoiled produce, emphasizing the potential health risks associated with consuming raw or minimally processed fruits and vegetables. The study underscores the need for improved hygiene practices during handling, storage, and sale, as well as the implementation of regular microbiological monitoring to ensure food safety in local markets.

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Fruits and vegetables are fundamental components of a healthy diet, offering a rich supply of nutrients such as fiber, vitamins, and essential minerals critical for maintaining overall health (Kaparapu *et al.*, 2020). Their consumption has been associated with numerous health benefits, including a reduced risk of acute and chronic conditions like cardiovascular diseases, cancer, and osteoporosis (Hodder *et al*., 2020). Global health organizations emphasize the importance of daily fruit and vegetable consumption to promote well-being and prevent diseases (Aune *et al.*, 2017). Despite their nutritional benefits, fruits and vegetables are highly susceptible to microbial contamination due to exposure to soil, dust, water, and human handling during harvest and post-harvest processing. These factors often lead to the presence of diverse microorganisms, including both plant and human pathogens (Osafo *et al.*, 2022). The risk of contamination is heightened when these produce are consumed raw or with minimal processing, which can expose consumers to pathogenic microorganisms (WHO, 2015). Contamination can arise from

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inadequate post-harvest handling, poor transportation, and improper packaging practices (Adams and Moss, 2018). Consequently, fruits and vegetables can act as vectors for foodborne pathogens, posing significant public health concerns (Altieri and Nicholls, 2017). In developing countries like Nigeria, the use of untreated wastewater and manure as fertilizers in the cultivation of fruits and vegetables is a major source of contamination (Amoah *et al*., 2009). The risks are exacerbated when produce is consumed raw or insufficiently cooked, as this may fail to eliminate all pathogens (Al-Tayyar *et al.,* 2020). As a result, outbreaks of foodborne illnesses linked to fresh or minimally processed fruits and vegetables have become increasingly common (Degaga *et al.,* 2022). Additionally, many street and market vendors who sell ready-to-eat fruits and vegetables in Nigeria operate without adequate food safety oversight. This lack of regulation increases the likelihood of contamination with pathogenic organisms such as *Escherichia coli*, *Salmonella spp.*, and other parasites (Oladele *et al.,* 2022). For instance, a study conducted in Oja-Oba Market, Ilorin, Nigeria, identified harmful bacteria, including *Escherichia spp.*, *Enterobacter spp.*, *Staphylococcus spp.*, *Salmonella spp.*, *Proteus spp.*, and *Shigella spp.*, in ready-to-eat fruits and vegetables (Ajiboye, 2021). This underscores the widespread microbial contamination in local markets. In markets like the Old Market in Patigi, Kwara State, where fresh produce is publicly displayed, the risk of contamination is compounded by environmental pollutants such as storm dust and stagnant water due to poor drainage. Despite these concerns, there is a paucity of research on the microbiological safety of fruits and vegetables sold in such markets. Thus, the objective of this paper is to isolate and identify pathogenic bacteria associated with Tomatoes (*Solanum lycopersicum*), bananas (*Musa spp*.), spinach (*Spinacia oleracea*), and okra (*Abelmoschus esculentus*) commonly sold at Old Market, Patigi, Kwara State, Nigeria

MATERIAL AND METHODS

Sample Collection: Two types of fruits, Tomatoes (*Solanum lycopersicum*) and Bananas (*Musa spp*.), together with vegetables of two different sorts, Spinach (*Spinacia oleracea*) and Okra (*Abelmoschus esculentus*), were sourced from four separate stationary sellers at the old market of Patigi. Fruits and vegetables collected were enclosed in sterile polyethylene bags and promptly transferred to the laboratory to preserve their freshness.

Preparation of Culture Media and Samples: In this study, media such as MacConkey Agar, Nutrient Agar, and Sabouraud Dextrose Agar were prepared following the manufacturer's instructions. The media were dissolved in distilled water, sterilized at 121°C for 15 minutes in an autoclave, cooled to 45°C, and poured into petri dishes to solidify. For sample preparation, 10 grams of fruit or vegetable were weighed, homogenized with a sterile pestle, and mixed with 90 ml of sterile distilled water in a conical flask. The mixture was agitated to ensure uniformity.

Serial Dilution, Isolation, and Enumeration: Serial dilutions were performed by transferring 1 ml of the stock sample into 9 ml of sterilized distilled water across five test tubes, creating dilutions up to 1/100,000. For bacterial isolation, 0.1 ml of the 10^{-3} dilution was spread on agar plates, incubated at 37°C for 24-48 hours, and colonies were counted to determine total bacterial load (cfu/ml). Pure cultures of isolates were obtained through sub-culturing, stored at 4°C, and characterized based on macroscopic and biochemical properties using the Karoki *et al.,* (2018) methodology.

Identification of Bacterial Isolates : The identification of bacterial isolates was conducted following the methodology outlined by Ajiboye, (2021), utilizing the Gram stain technique and biochemical tests including catalase, indole, citrate utilization, oxidase, methyl red, and Voges-Proskauer tests.

The catalase test involves the application of three (3) percent hyd rogen peroxide (H_2O_2) onto a clean, grease-free slide. A smear of a loopful of bacteria was prepared. Bubbles were found for each bacterial isolate.

Indole Test: A peptone broth was made by measuring 15 ml of peptone broth into a test tube and sterilized in an autoclave at 121℃ for 15 minutes under 15 psi pressure. A loop containing bacterial culture was put into broth and incubated for 24 to 48 hours at 37 ℃. Two drops of Kovac's reagent were added to the test tubes and stirred following sterilization.

The oxidase test involves placing two drops of oxidase reagent onto Whatman filter paper, followed by creating a smear of a 24-hour-old bacterial culture from a nutrient agar plate. The emergence of bubbles or effervescence indicated a positive result.

The Citrate Utilization Test involves the combination of 2 grams of Sodium Citrate, 5 grams of Sodium Chloride, 1 gram of Dipotassium Phosphate, 1 gram of Ammonium Dihydrogen Phosphate, 0.08 grams of Bromothymol Blue, 0.2 grams of Magnesium

Sulphate, and 15 grams of agar, followed by the addition of 1000 milliliters of sterile distilled water to the mixture. The pH was adjusted to 6.9, and mild heat was given to dissolve the agar. Approximately 3-4 cc will be collected in glass containers and sterilized at 121 ℃ for 15 minutes in an autoclave. The cooled medium was permitted to solidify in slant bottles, and inocula were applied to the surface of the slant. The bacterial culture was injected into a fresh sterile broth medium and cultured at 37 ℃ for 48 hours for the Methyl Red Test. A sterile pipette will deliver 5 drops of Methyl red reagent into the broth culture, and a color change will be seen.

The Voges-Proskauer Test involved the preparation of a 5% α-naphthol solution in ethanol and a 40% sodium hydroxide solution in deionized water. MR-VP broth was made, and 5 ml was distributed into several test tubes, which will be sterilized at 121 ℃ for 15 minutes using an autoclave. The medium was permitted to reach room temperature. Inoculum from new culture media was added to several test tubes and thereafter cultured alongside the control at 37 ℃ for 48 hours. Approximately 2.5 ml of culture was transferred into a sterile culture tube, followed by the addition of five drops of methyl red reagent. The test organism was compared with the control, and a color shift was noted.

The Nitrate Reduction Test involved placing Durham tubes in a test tube, into which 15 ml of nitrate broth was made and delivered. This will be subjected to autoclaving at 121 ℃ for 15 minutes for sterilization. The bacterial suspension will be injected into sterile broth and incubated at 37 ℃ for 24 hours. Gas formation was noted as an indication of a positive result. Six drops of Nitrite reagent A and six drops of Nitrite reagent B were produced and dispensed into the test tubes. Observations of color shifts were documented.

The Coagulase Test involved placing a few drops of physiological saline on two separate grease-free slides, onto which a loop of bacterial isolate was emulsified to create two suspensions. A drop of human plasma was obtained using a sterile Pasteur pipette and gently blended on the slides. The two slides were monitored for clumping for 5 to 10 minutes to ascertain a favorable outcome.

RESULTS AND DISCUSTION

Enumeration and isolation of bacteria isolates: The results of bacterial enumeration in the fresh fruits and vegetables are presented in table 1. The findings reveal that fresh tomato has the highest bacterial load of 2.8×10^4 cfu/ml while fresh okra has the least value of 0.7×10^4 cfu/ml while Table 2 shows the bacteria enumeration of spoiled fruits and vegetables sample, the spoiled banana has the highest bacterial load of 7.0×10^4 cfu/ml while the okra isolate has the least value of 3.3×10^4 cfu/ml.

Table 1: Total Bacteria Count on Nutrient Agar for fresh fruits and vegetables sold at old market, patigi.

Isolate	Dilution factor	Number of colonies	(cfu/ml)
Fresh banana A	10^3	18	1.8×10^4
Fresh tomato B	10^3	28	2.8×10^4
Fresh spinarch C	10^3	19	1.8×10^4
Fresh okra D	10^3	7	0.7×10^4

Table 2: Total Bacteria Count on Nutrient Agar for spoiled fruits and vegetables sold at old market, patigi.

Sample/Isolates	Dilution factor	Number of colonies	(cfu/ml)
spoil banana A	10^3	70	7.0×10^4
spoil tomato B	10^3	41	4.1×10^4
spoil spinarch C	10^3	52	5.2×10^4
spoil okra D	10^3	33	3.3×10^4

Table 3: colony morphology of bacterial isolate

Isolate Code	Colony Shape	Surface Appearance	Pigmentation	Edges	Arrangement
A	Round, flat	Iridescent	Non-pigmented	Irregular	-
B	Mucoid	Smooth	Non-pigmented	Smooth	Singly arranged
C	Circular, convex	Smooth, moist	Non-pigmented	Smooth, outer ring	-
D	Circular, rough	Opaque	White or slightly yellow	Jagged	-

Biochemical reaction of the Bacterial Isolate: The biochemical reactions test indicate the presence of *Enterobacter aerogenes, Klebsiella sp., Staphylococcus aureus, and Bacillus subtilis* as shown in Table 3.

Table 3: Biochemical Reaction of Bacterial from Ready to Eat Fruits and Vegetable sold at old market, patigi.

Isolate	Gram Reaction	Shape	Ca	Ox	Cit	Gas	Ind	Sp	U	MR	VP	Glu	Lac	Suspected Organism
A	-	Rod	+	+	+	+	-	-	+	+	-	+	+	*Enterobacter aerogenes*
B	-	Rod	+	-	+	-	-	-	+	-	+	+	-	*Klebsiella sp.*
C	+	Cocci	+	-	-	-	-	-	-	+	+	+	-	*Staphylococcus aureus*
D	+	Rod	+	-	+	+	+	+	+	-	-	+	+	*Bacillus subtilis*

Colony Morphology of Bacterial Isolates: Four bacterial isolates exhibited distinct colony characteristics. Isolate A was round and flat with an iridescent surface and irregular edges, while Isolate B had a mucoid, smooth colony with smooth edges, indicating potential capsular presence. Isolate C

showed circular, convex colonies with smooth edges and an outer ring, and Isolate D had rough, opaque colonies with jagged edges and slight yellow pigmentation. These morphological differences suggest variations in their ecological roles or pathogenicity.

Prevalence of bacterial species: The result for the prevalence of bacterial species in the fruits and vegetables sold in old market,patigi is presented in Table 4. The result showed that, tomato and spinach had the highest number of bacterial species with *Klebsiella sp.* as the most prevalent in all the fruits with 66.66%, 33.33% and 33.33% in banana, tomato and spinach respectively.

Table 4: Prevalence of bacterial species in fruits and vegetables sold at old market, patigi.

isolates	Bacterial sp	frequency	percentage
banana	*Klebsiella sp.*	2	66.67
	Bacillus subtilis	1	33.33
	total	3	100
tomato	*Enterobacter aerogenes*	1	16.67
	Klebsiella sp.	2	33.33
	Staphylococcus aureus	1	16.67
	Bacillus subtilis	2	33.33
	total	6	100
spinach	*Enterobacter aerogenes*	2	33.33
	Klebsiella sp.	2	33.33
	Staphylococcus aureus	1	16.67
	Bacillus subtilis	1	16.67
	total	6	100
Okra	*Bacillus subtilis*	2	100
	total	2	100

Keys: A: Bacterial isolate on banana; B: bacterial isolate on tomato; C: Bacterial isolate on spinach; D: bacterial isolate on okra; +:Positive; -:Negative; CT: Citrate test; VP: Voges-Proskauer; OX: Oxidase; CAT: Catalase; MOT: Motility' MR: Methyl red; IND: Indole

The findings from this study provide valuable insights into the bacterial loads associated with both fresh and spoiled fruits and vegetables sold in Old Market, Patigi. As presented in Table 1, fresh tomatoes exhibited the highest bacterial load of 2.8 x 10^4 cfu/ml, which could be attributed to various factors, including improper handling, suboptimal storage conditions, and the natural micro flora of the produce. In contrast, fresh okra had the lowest bacterial load of 0.7 x 10^4 cfu/ml, suggesting its physical characteristics, such as texture and surface area, might offer some protection against microbial adhesion. For spoiled produce (Table 2), bananas showed the highest bacterial load of 7.0 x 10^4 cfu/ml, reinforcing the association between spoilage and the proliferation of microorganisms due to tissue breakdown and nutrient availability. Conversely, spoiled okra exhibited a lower bacterial load of 3.3 x 10^4 cfu/ml, which may reflect differences in composition, moisture content, or spoilage dynamics. These findings align with the study of Ajiboye, (2021), who observed high bacterial loads in selected ready-to-eat fruits and vegetables, emphasizing the impact of handling and environmental factors on microbial contamination.

Biochemical tests (Table 3) identified *Enterobacter aerogenes*, *Klebsiella* sp., *Staphylococcus aureus*, and *Bacillus subtilis* among the isolates, supported by Gram reactions and morphological characteristics. *Staphylococcus aureus* is a well-known foodborne pathogen, while *Klebsiella* sp. and *Enterobacter aerogenes* are opportunistic pathogens frequently implicated in environmental and food contamination. These findings are consistent with Badri *et al.* (2022), who reported similar bacterial contaminants in tomatoes from South-Eastern Nigeria, with water quality and handling practices cited as potential sources of contamination. Further comparison with the study of Eni *et al.*, (2010), which investigated the microbial quality of fruits and vegetables from various sources in Sango-Ota, Ogun State, revealed the prevalence of *Escherichia coli* (4.2%), *Actinomycetes* (4.2%), *Salmonella* spp. (12.5%), *Klebsiella* spp. (12.5%), *Staphylococcus* spp. (12.5%), and *Staphylococcus aureus* (29.2%). Similarly, our study identified *Klebsiella* sp. as the most prevalent species, detected in banana (66.67%), tomato (33.33%), and spinach (33.33%), highlighting the significance of hygiene during handling and sales. Interestingly, *Bacillus subtilis* was the sole isolate from okra, with a prevalence of 100%, which may be attributed to environmental factors or handling practices unique to this vegetable. This emphasizes the need for stricter food safety protocols, including better hygiene practices, proper washing techniques, and adequate storage conditions, to reduce microbial contamination in fresh produce and protect consumer health. *Conclusion:* This study highlights the significant microbial contamination present in both selected fresh and spoiled fruits and vegetables sold at Old Market, Patigi, Kwara State. The identification of pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp.*, and *Staphylococcus aureus* underscores the public health risks associated with consuming these produce items, particularly when they are minimally processed. The findings reveal the need for improved hygiene during handling, storage, and sales to mitigate contamination. Additionally, regular microbiological monitoring and public education on food safety practices are recommended to safeguard consumers' health. Addressing these concerns is critical,

particularly in local Nigerian markets where regulatory oversight may be limited, thereby contributing to the overall improvement of food safety standards in the country.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors

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