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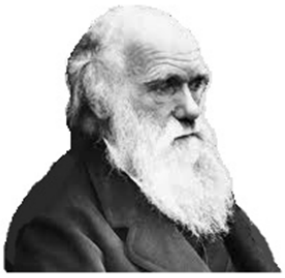

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Diversity of Microorganisms Associated with Deteriorated Tomatoes Sold by Vendors in Major Markets in Owerri

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ABSTRACT

Tomatoes hold considerable significance in Nigeria due to their substantial contributions to the employment sector, income generation for cultivators, and satisfaction of domestic and nutritional food requirements. Nonetheless, postharvest deterioration of a considerable quantity of tomato produce occurs in Nigeria as a result of the growth of spoilage organisms. The diversity of microorganisms associated with the deterioration of tomato fruits sold by vendors in four major markets in Owerri was analyzed. One hundred and twenty samples were randomly drawn from the various markets and subjected to standard microbiological techniques Total viable count (2.6×10^4 CFU/g), total coliform count (4.2×10^4 CFU/g), and total fungi count (7.0×10^5 CFU/g) were highest from deteriorated tomato samples analyzed from the relief market. A total of six bacteria genera belonging to *Micrococcus* sp., *Klebsiella* sp., *Escherichia coli*, *Proteus* sp., *Bacillus* sp., and *Staphylococcus* spp. were identified. *Staphylococcus* spp (31.75%) was the most predominant bacteria isolate associated with the deteriorated tomato samples. This was followed by *Escherichia coli* 4 (25%), *Bacillus* sp. 3 (18.75%), *Proteus* sp. 2 (12.5%), *Enterobacter* sp. 1 (6.25%), and *Micrococcus* sp. *Penicillium* sp., *Aspergillus* sp., and Rhizopus sp. were also isolated from the deteriorated tomatoes, with *Rhizopus* sp. being the most predominant isolate 6 (40%) while *Aspergillus* sp. 4 (26.7%) was the least predominant isolate. The isolation of potential pathogens from deteriorated tomatoes sold by vendors in the four major markets in Owerri represents a significant health risk to consumers. A robust measure to combat post-harvest deterioration of tomatoes should be put in place by government agencies at all levels. Additionally, public enlightenment and awareness campaigns on the consequences of consuming deteriorated tomatoes should be encouraged.

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***Keywords*:** Diversity, Deterioration, Tomatoes, Microorganisms, Bacteria, Fungi, pathogens

1. INTRODUCTION

The tomato (*Lycopersicum esculentum*) is a sort of fleshy fruit sometimes referred to as a vegetable. Its soft pulp and thin skin distinguish it from many seeds [1]. The significance of tomatoes globally is steadily growing due to their consumption as a fresh crop, their prominent role in many prepared dishes, and their potential as valuable resources for studying the basic principles of plant growth and development [2]. Tomato fruits are a fundamental component of the human diet.

These entities provide fundamental sustenance, vital vitamins, and trace elements with nutritional value.

Furthermore, it has a significant influence on enhancing the taste and desirability of food [3]. The fruits are used in several ways, whether fresh or prepared. Tomato fruits are used as medicine, nutrient supplements, flavouring ingredients, detoxification, and human system cleansers [4].

Tomatoes contain lycopene, one of the most potent antioxidants in nature. It has advantageous effects in combating several disorders, including cancer [5]. From an economic standpoint, tomatoes are the most valuable edible vegetables [1] and are widely available in most places worldwide. Tomato fruits are known to possess a diverse array of nutrients and vitamins. [6] asserts that environmental variables such as pH levels, trace metal ion concentrations, and oxygen significantly impact the system's stability.

Nevertheless, despite the advantageous nature of tomato fruit for human consumption, there is a substantial post-harvest loss and deterioration due to microbial activity, especially in Nigeria, where storage facilities are grossly inadequate [2].

One of the microorganisms that may infect tomato fruits is a fungal plant pathogen, potentially resulting in significant fruit loss [7]. There is a greater risk of bacterial contamination because highly toxic substances and bacterial spores might not be found until a food poisoning outbreak [8]

Additionally, the growth of fungi on the fruits increases the chances of mycotoxin presence on these fruits. Mycotoxins have been shown to have carcinogenic, nephrotoxic, teratogenic, and immunosuppressive properties ([9]. Despite the health challenges associated with the consumption of deteriorated tomatoes, they are still widely patronized in Nigerian local markets among middle- and low-income earners due to their reduced cost [10]. Consuming these deteriorated tomatoes could be potentially harmful, especially when not adequately cooked.

The proliferation of fungi in tomatoes is occasioned by favourable factors such as maturity, ripeness, and highwater activity [11]. However, these factors also serve as optimum conditions for the production of mycotoxins [12].

Additionally, tomatoes permit the growth of other bacteria and yeasts, which may have stimulatory effects on the production of mycotoxins [12]. This study was therefore, designed to assess the microbial diversity associated with deteriorated tomatoes sold by vendors in major markets in Owerri.

2. MATERIALS AND METHODS

2. 1. Sample collection and processing

One hundred and twenty (120) deteriorated tomato fruit samples were obtained from vendors in four markets: Akwakuma Market, Relief Market, Nkwor Orji Market, and Alaba Market. The samples were gathered in sterile containers in pre-chilled containers equipped with ice packs and conveyed to the laboratory for examination.

3. MICROBIOLOGICAL ANALYSIS

3. 1. Enumeration and isolation of microorganisms

Each deteriorated tomato sample was aseptically macerated in a sterile mortar and pestle. Aliquots of 1.0 ml were mixed with 9 ml of sterile distilled water, agitated properly, and serially diluted up to $10^{-6.}$ Aliquots of 0.1 ml were plated on nutrient and Saboraud dextrose agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) based on standard microbiological procedures. Plates were incubated at 37 °C for 24 and 72 hours for bacteria and fungi. The colonies were enumerated using a colony counter, and only colonies appearing on plates with a colony count ranging from two to ten were selected for enumeration. The isolates were purified using streaking on plate count agar plates.

3. 2. Identification of bacterial isolates

Bacterial isolates were identified based on macroscopically, microscopically and biochemical characteristics. Gram staining, catalase test, oxidase, motility, citrate utilization, vogue Proskauer, methyl red test, indole test, and sugar fermentation test were carried out.

3. 3. Identification of fungi isolates

Fungi isolates were repeatedly sub cultured by streaking on freshly prepared potato dextrose agar plates until pure colonies were obtained [13]. The isolates were identified based on their cultural and biochemical properties, according to [13].

4. RESULT

4. 1. Enumeration of microorganisms from deteriorated tomatoes

The results of enumeration of microorganisms from the deteriorated tomato samples is presented in Table 1. Results showed that total viable count (TVC) ranged from $1.4 \times 10^4 – 2.6 \times 10^4$ CFU/g, while the total fungal count ranged from $3.0 \times 10^5 – 7.0 \times 10^5$ CFU/g. The total coliform count ranged from $1.6 \times 10^4 – 4.2 \times 10^4$ CFU/g (Table 1).

Table 1. Enumeration of microorganisms from deteriorated tomatoes samples.

Samples	Total viable count	Total coliform count	Total fungal counts
A	1.8×10^4	3.1×10^4	3.0×10^5

B	2.6×10^4	4.2×10^4	7.0×10^5
C	1.4×10^4	2.3×10^4	3.2×10^5
D	2.1×10^4	1.6×10^4	4.8×10^5

Keys: A: Akwukuma Market; B: Relief Market; C: Nkwor Orji Market; D: Alaba Market

4. 2. Identification of microorganisms from deteriorated tomatoes

Based on the morphological, cultural and biochemical characteristics, bacterial isolates were identified as *Micrococcus* sp., *Klebsiella* sp., *Escherichia coli*, *Proteus* sp., *Bacillus* sp., and *Staphylococcus* sp. (Table 2 and 3) while *Penicillum* sp., *Aspergillus* sp. and *Rhizopus* sp. were identified as fungi isolates (Table 4)

Frequency of occurrence of bacteria associated with deteriorated tomatoes samples *Staphylococcus aureus* (31.75%) was the most predominant bacteria isolates associated with the deteriorated tomato samples This was followed by *Echerichia coli* 4 (25%), *Bacillus* sp. 3 (18.75%), *Proteus* sp. 2 (12.5%), *Enterobacter* sp. 1 (6.25%) and *Micrococcus* sp. 1 (6.25 (Table 5). For fungi isolates, *Rhizopus* sp predominated 6 (40%) while *Aspergillus* sp. 4 (26.7%) was the least predominant (Table 6).

Table 2. Colonial and morphological characteristics of bacteria isolates from deteriorated tomatoes

S/N	Colonial morphology	Gram reaction	Shape and arrangement	Endospore	Motility	Suspected organisms
1	White, flat, large, smooth, opaque, moist and glistering	-	Short rods in scattered arrangement	-	+	*Escherichia coli*
2	Yellow, circular, smooth and soft	+	Cocci in irregular clusters			*Micrococcus* sp.
3	Thin, blue-grey, spreading growth	+	Short rods in scattered arrangement	-	+	*Proteus* sp.
4	White, glistening, thick, mucoid with dark centre and light egde	-	Short rods in scattered arrangement			*Enterobacter* sp.

5	White, dry, attached, spreading with crenate margin	+	Long rods with round edges in scattered arrangement	+	+	*Bacillus* sp.
6	Golden yellow, large, circular, convex, smooth, shiny and opaque	+	Cocci in bundles	+	+	*Staphylococcus aurus* sp.

Key: - = absent; + = present

Table 3. Biochemical identification of bacteria isolates from tomatoes.

Biochemical tests	*E. coli*	*Micrococcus* sp	*Proteus* sp.	*Enterobacter* sp.	*Bacillus* sp.	*Staphylococcus* sp.	*Klebsiella* sp.
Indole test	+	-	+	-	-	-	-
Methyl red	+	-	+	-	-	+	-
Voges proskauer	-	-	-	+	+	+	+
Citrate utilization	-	+	-	+	+	+	+
Catalase	+	-	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Urease	-	+	+	-	-	-	-
H_2S	-	-	+	-	-	-	-
Glucose	AG	-	AG	AG	A	A	AG
Sucrose	A	-	AG	AG	A	A	AG
Lactose	AG	-	-	AG	-	A	AG
Mannitol	-	-	-	AG	A	A	AG

KEYS: AG = Acid and Gas, A = Acid, + = Present, - = absent

Table 4. Colonial and morphological characteristics of fungi isolated from tomatoes

Microscopic characteristics	Morphological characteristics	Suspected organism
Upright conidiophores with striped smooth wall. Conidia are one celled vesicle septate and collumella	White base with black conidiophores which make it appear black in colour	*Aspergillus* sp.
Presence of stolons and pigmented rhizoids formation of sporangiosphores	On culture plates they were initially white, covering the agar surface with dense cottony growth but later became grey- yellowish brown colour with spoulation	*Rhizopus* sp.
The conidiophores also gives rise phialides which were thick walled and borne lliptical conidia which were globose with greenish smooth wall, branched and septate, slightly long, smooth and swollen	The colonies appeared pale green with pheripheral white colour, becoming bluish grey with maturation.	*Penicillium* sp.

Table 5. Percentage frequency of occurrence of microorganisms associated with deteriorated tomatoes samples

Bacteria	No of Occurrence	% Occurrence
E. coli	4	25
Micrococcus sp.	1	6.25
Proteus sp.	2	12.5
Staphylococcus sp	5	31.25
Bacillus sp.	3	18.75
Enterobacter sp.	1	6.25
Total	16	100

Table 6. Frequency of Occurrence of Fungi Associated with Tomato Samples

Fungi	No of occurrence	% of occurrence
Aspergillus sp.	4	26.7
Rhizopus sp.	6	40
Penicillum sp.	5	33.3
Total	15	100

5. DISCUSSION

Tomatoes are of utmost importance in Nigeria as they contribute significantly to the country's employment sector, producers' income generation, and fulfil domestic and nutritional food needs. Nigeria currently ranks as Africa's second-largest producer of fresh tomatoes, contributing 10.8% to the region's overall production. Nigeria is also the largest consumer of tomatoes in Africa, estimated to consume 2.3 million tons annually, with a per capita consumption of 12 kg in 2016. However, many are subjected to deterioration due to poor storage facilities and adverse effects of environmental and biological factors. [14]

This present study evaluated the microbial diversity associated with deteriorated tomatoes sold in local markets in Owerri.

Samples from the relief market recorded the highest total viable count, coliform count, and fungi count. Results also show that total fungi counts were generally higher that total viable count in all the markets. This trend could be attributed to the high influx of buyers and the unhygienic environments [15]. The relief market is the biggest market among the market samples. Similar results have also been reported by [16] & [17]

The total fungi count was higher than the mean total bacteria count. This may be attributed to the ability of bacteria to produce inhibitory compounds that inhibit the growth of other bacteria. (Jay, 2000). However, this report contradicts the findings of [16], who reported a higher mean total bacteria count from their studies. A total of six (6) bacteria genera, including *Escherichia coli, Micrococcus spp., Enterobacter spp., Bacillus spp., Staphylococcus spp., and Klebsiella* spp., were identified. Coliforms in the deteriorated samples, including Escherichia coli, *Enterobacter*, and *Klebsiella* sp., suggest contamination from feacal matter.

E. coli, Enterobacter, and *Klebsiella* sp inhabit the human gut and the intestines of humans and animals. While most of these organisms are non-pathogenic, several strains can potentially induce gastroenteritis, diarrhoea, urinary tract infections, endocarditis, and meningitis ([18].

The presence of these potential pathogens is of great significance to public health. *E. coli* O157:H7 produces a toxin known as Shiga toxin, which can cause bloody diarrhoea, renal failure, and potential death [19].

Staphylococcus aureus was the most predominant organism isolated from the deteriorated tomato fruit samples. Staphylococcus, a microflora of the skin, nasal cavity, and throat, suggests inadequate hygiene practices by the vendors.

Suppose *Staphylococcus aureus* is left to proliferate in food. In that case, it can generate a toxin that may cause illness [20]. [21] opined that *Staphylococcus aureus* is the primary cause of foodborne illnesses.

Penicillium sp., *Rhizopus* sp., and *Aspergillus* sp. were isolated from the deteriorated tomatoes, with Aspergillus being the most predominant organism. This report agrees with the findings of [22] implicated *Aspergillus* sp. and *Penicillium* sp. in deteriorated tomatoes. These are predominantly soil microorganisms whose spores can quickly spread through the air and thus infect exposed tomatoes and other farm tools.

Rhizopus stolonifera has been implicated as a soft rot pathogen of tomatoes cultivated in Nigeria. [23] has also implicated. *Rhizopus stolonifer* as a soft-rot pathogen of tomatoes in Nigeria. The fungus's mycelium can invade neighboring fruits by exploiting mechanical injuries and natural apertures, forming a cluster of mould development on infected fruits [24].

In addition to inflicting significant economic damage, several fungal species can excrete extracellular toxigenic metabolites, such as mycotoxins, posing a possible safety concern to people [25]. Furthermore, tomatoes and vegetables have often acted as carriers for pathogens and have been associated with several outbreaks of foodborne illnesses.

6. CONCLUSION

This investigation has identified the contamination of possible pathogens in deteriorated tomatoes sold in major markets in Owerri with a significant potential health hazard. Therefore, implementing efficient measures to manage bacteria, fungi, plant pathogens, and contaminants in tomato plants should be brought to the front burner and sustained. This measure t will significantly decrease their deterioration, enhance the quality of tomato fruits available to consumers, and mitigate the health risks associated with consuming contaminated fruits and vegetables in Nigeria.

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