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Bacteriological Evaluation of Tomatoes Purchased From Ilesha, Osun State

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ABSTRACT

This study will look at microorganisms identified in tomatoes purchased in Ilesha, Osun State. The microbial degradation of tomato fruits lowers their market value and reduces their nutritional content. Ilesha in Osun State provided a total of five (5) tomato samples. All of the samples were collected aseptically in sterile universal containers and sent to the lab for examination promptly. Each tomato sample was weighed and homogenized in 90mL sterile distilled water, then blended in a sterile blender, and 1 mL of the homogenate was constituted in 9 mL sterile peptone water. The bacteria isolates were identified using Gram staining and other biochemical testing. The biochemical tests used to characterize the microorganisms further. The total viable count in colony forming units per milliliter (CFU/mL) is the outcome of the total viable counts. Sample T1 has the highest overall viable plate count of 12.0×10-4, followed by sample T4, which has 5.7×10-4, sample T3, which has 3.6×10-4, sample T5, which has 2.5×10-4, and sample T2, which has 2.4×10-4. Except for T2a, T2b, and T5 which are Gram positive cocci, all bacterial isolates with sample codes T1, T3, and T4 are rod-shaped and gram negative. Erwinia sp, Lactobacillus sp, Micrococcus sp, Proteus sp, Pseudomonas sp, Pseudomonas sp, and Staphylococcus sp are among the microbes that could be identified. This study discovered that tomato fruits are connected with various opportunistic pathogenic bacteria, posing a considerable risk to consumers, particularly the illiterate majority, who are unaware of the risks and can eat tomato fruits without washing them.

1. INTRODUCTION

Tomato (Solanum lycopersicum), along with potato (Solanum tuberosum), hot pepper (Capsicum frutescens), pepper (Capsicum annum), and eggplant (Solanum melongena), is a significant crop in the Solanaceae family (Shah *et al*., 2017). It is the most widely planted plant on the planet, accounting for more than 14% of global fruit production (FAO, 2011). China is the world's biggest tomato producer, followed by India, the United States, Turkey, Egypt, Iran, and Italy, which account for more than 80% of global tomato production, according to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (FAO, 2011). Egypt is the only African country in the top 10 tomato growers in the world (DAFF, 2016).

Tomatoes are the second most significant and popular crop in South Africa, behind potatoes,

from the Solanaceae family, and one of the most commonly marketed plants on both local and international markets (DAFF, 2016). Furthermore, according to the Department of Agriculture, Forestry and Fisheries (DAFF), 75 percent of South Africa's tomatoes are grown in Limpopo province's northern parts, with the remaining 25 percent grown between Mpumalanga province's Onderberg area and the Eastern Cape Province's border area (DAFF, 2016).

Tomatoes are a popular fruit that can be eaten both fresh and cooked. *Lycopersicum esculentum* is the botanical name for this plant, which belongs to the solanaceae family. Vitamins B, C, and E are abundant in this fruit. Carbohydrates like fructose and glucose, as well as trace minerals like iron, copper, zinc, and dietary fiber, are all essential nutrients for human health. Tomatoes have a high water content, which makes them more prone to microbial deterioration (Obunkwu *et al*, 2018).

Tomatoes are highly important for their dietary needs, and they may be taken in a variety of ways: as a vegetable, as an ingredient in a variety of recipes and sauces, in stews, fruit juices, and uncooked in salads (Onuorah and Orji, 2015). Tomato rotting refers to the degradation of tomato quality caused by biological and physical processes. Taste, smell, look, and texture of the fruits may all alter as a result of these modifications (Onuorah and Orji, 2015).

According to estimates, nearly one-third of the produce is wasted before it reaches the consumer (Mbajiuka and Emmanuel, 2014). This loss has been attributed to a range of reasons, including physical (mechanical breakage, bruising) and microbiological (fungi and bacteria) damages (Onuorah and Orji, 2015). The microbial degradation of tomato fruits lowers their market value and reduces their nutritional content. Contamination of tomato fruits with mycotoxins causes aflatoxins to be produced in humans after inhalation or ingestion, resulting in food poisoning (Bello *et al*., 2016).

Some research has been done to discover bacteria that are linked to tomato rotting. *Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis*, and *Staphylococcus aureus* were isolated from spoiled tomatoes in Benin City by Wogu and Ofuase (2014). In Lagos State, Nigeria, a similar study found significant amounts of Staphylococcus sp, Bacillus sp, and Escherichia coli (Ogundipe *et al*., 2012). Rhizopus sp have been linked to tomato rotting, according to Akinmusire (2011).

Tomatoes' susceptibility to microbial deterioration is partly due to their high moisture content. Freshly harvested tomato fruits are stored, transported, and sold in wooden boxes and baskets in northern Nigeria. These baskets are frequently used until germs and/or fungal spores contaminate them. Pathogenic inoculums on these wooden boxes and baskets can cause rotting when they come into touch with healthy tomato fruits, resulting in losses, which translates to a waste of resources, a fall in income, and, ultimately, a reduction in welfare for the farmers. Infected farm tools or transportation could also be a source of pathogenic inoculums.

Proper isolation and characterization of these organisms in tomatoes can greatly reduce the rotting of this perishable fruit, allowing producers and consumers to protect their veggies (tomatoes) as well as identify damaged tomatoes infected with fungi and bacteria. Tomatoes are one of the world's most popular and widely grown plants, as well as in Africa. In terms of the amount of vitamins and minerals it contributes to the diet, it is the world's second most important vegetable (Osemwegie *et al*., 2010). The goal of this study was to figure out which bacteria are linked to tomato rotting.

2. METHODOLOGY

2.1. Collection of samples

In Ilesha, Osun State, a total of five (5) tomato samples were collected. All of the samples were

taken aseptically in sterile universal containers and transported to the laboratory for examination right away.

2.3. Media preparation

Nutrient agar, mannitol salt agar, eosin methylene blue agar, MacConkey agar, salmonella-shigella agar, and peptone water were all made according to the manufacturer's instructions.

2.4. Isolation of bacterial from the tomatoes samples

Each tomato sample was weighed and homogenized in 90 mL of sterile distilled water, then blended in a sterile blender, and 1 mL of the homogenate was constituted in 9 mL of sterile peptone water. Then, using the pour plate technique, 0.1 mL of the last two dilutions (10^{-4} and 10^{-6}) were inoculated in triplicate on properly prepared media. The plates were then incubated for 24 hours at 37°C.

The plates were checked after incubation to see if there were any separate colonies. The colony counter was used to count colonies, which were quantified as colony forming units per gram (CFU/g) of sample homogenate. On nutritional agar, a total aerobic count was done, and Escherichia coli were counted on Eosin methylene blue agar.

According to Oranusi and Olorunfemi, Mannitol salt agar and MacConkey agar were used to count *Staphlococcus aureus* and non *E.coli* coliforms, respectively, while Salmonella Shigella agar was used to count Salmonella after 24 hours of pre-enrichment of sample homogenate in Selenite-Flouch (2011). Discrete colonies on various media were separated and purified through repeated subculturing on the same media. For further characterisation, pure colonies were kept on agar slants at 4^{o}C.

2.5. Purification and Maintenance of Isolates

Each distinct colony on a petri dish was transferred into plates containing newly prepared nutritional agar using a sterile inoculating loop and cultured at 37°C for 24-48 hours. The colonial morphologies (Cultural traits) of the isolates were documented after incubation and compared to descriptive features in Holt *et al* (1994). The isolates were then conserved on nutrient agar slants and kept at 4 degrees Celsius in the fridge.

2.6. Biochemical characterization and Identification of Isolates

The bacteria isolates were identified using the method developed by Oranusi *et al*. (2004). Catalase, mrthyl red, oxidase, citrate utilization, and coagulase and indole assays were among the biochemical tests utilized to further describe the bacteria. The gram negative isolates were also subjected to an oxidase test to determine whether they were oxidase positive or negative. The identities of coliforms and bacteria were then confirmed using the Bergy's Manual for Determinative Bacteriology's identification procedures (Holt *et al*., 1994)

2.6.1. Identification of Isolates

Cheesbrough's method was used to perform Gram staining and other biochemical assays (2006). Each bacterial isolate's colonial appearance on the plate was inspected and classified using the following criteria: color, shape, edge, elevation, surface, and opacity (Olutiola *et al*., 2000). Catalase, oxidase, indole, and coagulase tests were among the biochemical tests performed here.

2.6.2. Biochemical Characterization

2.6.2.1. Gram Staining

Gram staining was employed in this investigation to distinguish Gram positive and Gram negative bacterial isolates based on their capacity to preserve the primary (Gram positive bacterial) or secondary (Gram negative bacterial) stain (gram negative bacterial). A smear of the

test isolate was emulsified in a drop of sterile distilled water on a clean glass slide, forming a smooth suspension, air dried, heat fixed by passing the inoculated glass slide through the Bunsen burner flame for about 2-3 times, stained with the primary stain, crystal violet for 60 seconds, and then rinsed off in slow running tap water. It was then decolorized for about 5 seconds with 70% ethanol, followed by a heavy rinse with water to avoid decolorization.

Finally, the smear was counter stained with safranin for 60 seconds, rinsed with water, and allowed to air dry before a drop of immersion oil was applied to the smear and viewed under a microscope with oil immersion lenses. Cells of isolates that held the purple color of the primary stain, crystal violet, were labeled as gram-positive bacteria, whereas those that were unable to retain the color of the primary stain but stained with the pink color of the counter stain were labeled as gram-negative bacteria (Fawole and Oso, 2001).

2.6.2.2. Catalase test

In a test tube, 2-3 mL of hydrogen peroxide was purified. A colony of test organisms was obtained and immersed in hydrogen peroxide solution using a sterilized wooden or glass rod. The presence of bubbles indicated the presence of oxygen. The organism was catalase positive if bubbles were formed; if bubbles were not created, the organism was catalase negative.

2.6.2.3. Coagulase test

The test was carried out according to Barrow and Gelthan's instructions (1993). 0.1 mL of an 18-24 hour old broth culture of the tested organism was added to 0.5 mL of a 1:10 dilution of human plasma in saline, incubated at 37°C for 2-24 hours, and coagulation was assessed. A favorable outcome was suggested by the creation of a distinct clot.

2.6.2.4. Citrate test

In 100 mL of distilled water, 2.4 g of citrate agar was dissolved. 10 mL citrate medium was dispensed, covered, sterilized, and allowed to cool in a tilted posture in test tubes. The tubes were injected once over the surface with the 24-hour-old culture organisms. The transition from green to blue indicates that the citrate has been used (Cheesbrough, 2006).

2.6.2.5. Indole test

The test bacterial colony was injected in tryptophan broth and cultured for 24-28 hours at 37°C. Then Kovac's reagent (0.5 mL) was added. A positive result was obtained. After adding the reagent, a pink tinted rink appeared. Negative test: no color change after the addition of the reagent.

2.6.2.6. Methyl red (MR) test

5 mm glucose phosphate broth (1 g glucose, 0.5 percent KH_2PO_4, 0.5 percent peptone, and 100 mL distilled water) was poured and sterilized into clean test tubes. After inoculating the tubes with the isolated test organisms and incubating them at 37°C for 48 hours, a few drops of methyl red solution were given to each test, and the color change was noted. A good reaction is indicated by the color red (Olutiola *et al*., 2000).

2.6.2.7. Voges-proskaeur (VP) test

In clean test tubes, 5 mm of glucose phosphate broth (1 g glucose, 0.5 percent KH_2PO_4, 0.5 percent peptone, and 100 mL distilled water) was poured and sterilized. The test organisms were then put into the tubes, which were then incubated at 37 °C for 48 hours. Following incubation, 6 percent -naphthtol and 6 percent sodium hydroxide were added to around 1 mL of the isolated organisms' broth culture. Within 30 minutes, a bright red coloration signals a favorable reaction (Olutiola *et al*., 2000).

2.6.2.8. Hydrogen sulphide test

A little amount of 24 hour old culture was injected into triple sugar iron agar tubes that had already been prepared and inoculated at 37^{o}C for 48 hours. The tube was checked for hydrogen sulphide (black coloring) gas generation after the incubation period (Oyeleke and Manga, 2008).

2.7. Sugar fermentation

Peptone water medium with 1% fermentable sugar and 0.01 percent phenol red was used to make sugar indicator broth. Each test tube received about ten milliliters of sugar broth, and the Durham tube, which would trap any gas that was created, was gently inverted. The test tubes were autoclaved and inoculated with a loopful of 24 hour old culture of the test organisms, after which they were cultured for 2-7 days at 361 °C and acid and gas generation was monitored daily. The displacement of the medium in the Durham tube showed acid generation, while the yellow colour indicated gas production (Fawole and Oso, 2004).

3. RESULTS AND DISCUSSION

3.1. Results

Total viable counts in colony forming units per gram (cfu/ml) are shown in Table 1. Sample T1 has the highest total viable plate count of 12×10^{-4}, followed by sample T4, which has 5.7×10^{-4}, sample T3, which has 3.6×10^{-4}, sample T5, which has 2.5×10^{-4}, and sample T2, which has 2.4×10^{-4}.

Table 1: Total viable counts from samples of tomatoes obtained from Ilesha, Osun State

S/N	Sample Code	Total Viable Count
1	T_1	12×10^{-4}
2	T_2	2.4×10^{-4}
3	T_3	3.6×10^{-4}
4	T_4	5.7×10^{-4}
5	T_5	2.5×10^{-4}

Table 2: Gram Reaction of Bacterial isolated from Tomatoes purchased from ilesha, Osun State

S/N	Sample Code	Positive/Negative (+/-)	Shape
1	T_1	-	Rod
2	T_{2a}	+	Cocci
3	T_{2b}	+	Cocci
4	T_3	-	Rod
5	T_4	-	Rod
6	T_5	+	Cocci

Except for T2a, T2b, and T5 which are Gram positive Cocci f, all bacterial isolates with sample codes T1, T3, and T4 are rod-shaped and gram negative (Table 2).

Table 3: Biochemical characteristics and Sugar fermentation of bacterial isolated from Tomatoes obtained from Ilesha, Osun State

Isolate code	Cat	Coag	Cit	In	MR	VP	HS	GH	Lac	Glu	Suc	SP	AF	Probable Identity
T_1		-	+	+	-	+	-	+	+	+	+	-	-	*Erwinia sp*
T_{2a}	-	-	-	-	-	-	-	+	+	+	+	-	-	*Lactobacillus sp*

T_{2b}	+	+	+	-	-	+	-	-	+	+	+	-	-	*Micrococcus sp*
T_3	+	-	+	-	-	+	+	+	-	+	+	-	-	*Proteus sp*
T_4	+	-	-	+	+	-	+	+	+	+	+	-	-	*Pseudomonas sp*
T_5	+	+	-	-	+	+	-	-	A	A	A	-	-	*Staphlococcus sp*

Keys: Cat= catalase, Coag=Coagulase, Cit=Citrate, In=Indole, MR=Methyl Red, VP=Voges Prosekeaur, HS= Hydrogen sulphide, GH= Gas production, Lac=Lactose, Glu-Glucose, Suc-Sucrose, SP=Spore formation, AF=Acid fast, - = negative, + = positive

From Table 3 above, Probable identity of bacterial isolates were determined after biochemical and sugar fermentation test were conducted on isolates. The results of biochemical and sugar tests were compared with Bergy's manual for bacterial identification (Table 3). The six isolates were probably identify as *Erwinia sp, Lactobacillus sp, Micrococcus sp, Proteus sp, Pseudomonas sp* and *Staphlococcus sp*

3.2. Discussion

The study's overall viable counts are within acceptable limits, yet the value is considerable. The considerable microbial development observed for tomato fruits received from the market may be due to factors such as storage, transportation, packaging, and distribution (loading and off-loading) at various channels and selling outlets. This research backs up the findings of Baiyewu *et al*. (2007), who found that tomato fruits are frequently exhibited in baskets and on benches in supermarkets, exposing them to opportunistic microbes.

In a similar line, Beuchat (2002) found that throughout field cultivation, harvesting, post-harvest processing, storage, and distribution, tomato fruits' natural protective cover (epidermal layer) is hampered and contaminated. The germs' probable identities include *Erwinia sp, Lactobacillus sp, Micrococcus sp, Proteus sp, Pseudomonas sp, Pseudomonas sp*, and *Staphlococcus sp*, which agrees with prior findings by previous researchers that recovered these microorganisms from tomato fruits (Oyemaechi *et al*., 2014; Bello *et al*., 2016)

Erwinia sp. and *Staphylococcus aureus* were among the microorganisms found in the tomatoes. The bacteria recovered in this study are comparable to those isolated by Ogundipe *et al*. (2012) and Wogu and Ofuase (2014) as species linked to tomato rotting. The presence of bacterium species could be attributed to feacal contamination caused by farmers and/or sellers' poor hygiene procedures. According to Obunkwu *et al*., microbial contamination and development on tomatoes causes deterioration, lower sensory appeal, and shorter shelf life, resulting in product loss and wastage, which has considerable economic ramifications (2018).

The presence of enteric bacteria on tomato fruits could be attributed to the tomato fruits being exposed to feces-contaminated irrigation water as well as the organic manure utilized in the bioremediation process (Amoah *et al*, 2009). These enteric bacteria could be transported from manure to soil, then to tomato fruits. *Staphylococcus sp*. was recovered from all of the tomato samples tested; it is a well-known micro flora of human nostrils, skin, and hands. It could have come from the corpses of the traffickers or the contaminated air.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

Some of the frequent contaminating bacteria isolated from tomato fruits could have come from the farm during harvest, while others could have arrived as post-harvest contaminants, most

likely during storage or transit. Tomato spoiling is also caused by reckless handling of these fruits by traders and customers, which includes the use of direct river or stagnant water in washing and soaking of these fruits by market traders. This can lead to a situation where the fruits are more susceptible to infection.

Contamination is increased when dirty tables are used when rags are used to cover tables. Vendors' usage of soiled cases and baskets for loading tomatoes, as well as their pouring of these fruits on the floor as a method of preservation, result in easy contamination of these tomato fruits.

Contamination of these fruits is also accelerated by changing market conditions and changing environmental conditions. This study discovered that tomato fruits are home to a variety of opportunistic pathogenic microbes, posing a significant risk to consumers, particularly the illiterate, who are unaware of the dangers and can eat tomato fruits without washing them.

4.2. Recommendation

It is recommended that;

1. Thorough washing of harvested tomatoes with clean or chlorinated water, adequate cleaning and sanitation of storage facilities, disinfection of packaging containers, and proper handling of the crop during harvest should all be done to avoid bruises, scars, or other mechanical injuries.
2. It is recommended that bacterial and fungal growth be inhibited by lowering storage temperatures to less than 100^{o}C but not freezing, and that appropriate antimicrobial agents be used when kept by drying.
3. The presence of microorganisms on the surface of tomato fruits reduces the shelf life of the fruit. It is therefore recommended that tomato fruits be surface sterilized after harvesting or before eating, especially if the fruits are to be consumed raw.
4. This study also discovered that lesions on the surface of tomato fruits can act as entry points for these microbial infections. Better harvesting procedures that limit cuts could assist to reduce the health risks connected with raw tomato eating.
5. Tomatoes can be infected by germs that have decomposed in contaminated air, insects, or animals. It is critical to keep rats, birds, and insects out of packaging and storage spaces since they can distribute or potentially be the source of harmful bacteria.
6. Sorting injured fruits thoroughly is critical to preventing post-harvest deterioration later on.
7. Tomato-loading baskets should be inspected for cleanliness and, if necessary, cleaned and sanitized before being loaded.
8. Finally, good sanitation practices should be followed from harvest to distribution to customers.

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