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MICROBIAL ASSESSMENT OF TOMATOES (*LYCOPERSICON ESCULENTUM*) SOLD AT SOME CENTRAL MARKETS IN MAIDUGURI BORNO STATE, NIGERIA

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Abstract

Lycopersicon esculentum (tomato) is a fleshy berry that is highly perishable and prone to microbial spoilage. The presence of some microorganisms of public health significance makes it a potential health hazard to consumers. There is therefore the need to determine the food safety and public health implications of some of the microorganisms present in it. Thirty (30) samples of tomato were obtained from three (3) major produce markets in Maiduguri. The average pH values of the tomato samples ranged from 3.30 to 4.60. While the average moisture content of the tomato samples was between 93.6% and 95.2%. The viable aerobic bacterial count of all the samples ranges from $1.38x10^6$cfu/g to $4.80x10^6$cfu/g. A total of 20 isolates were obtained from the tomato samples. *Escherichia coli* (50%), *Enterobacter* spp (30%), *Klebsiella* spp. (20%) were the bacteria isolated from the tomato samples. The results showed that the freshness and hardness of tomato samples were important factors which determine the types of bacteria found on them. As some of the bacterial genera identified are potential food-borne pathogens that could pose some public health challenges, proper handling and adequate cooking before consumption is suggested.

Keywords: *Lycopersicon esculentum* (tomato), public health, Ph, and *Escherichia coli.*

Introduction

In our contemporary environment, there is a higher risk of bacterial contamination due to new trends in trade patterns, agriculture, different food processing techniques, and dietary practices (Smolin and Grosvenor, 2003). Although vegetables are essential sources of the nutrients the body needs, eating unwholesome or contaminated veggies can result in food-borne illnesses (Allende *et al*., 2011). According to research, the renewed interest in vegetable eating as a key source of vitamins and other minerals has led to an increase in foodborne diseases recently (Abadia *et al.,* 2008). A popularly cultivated and consumed perishable vegetable around the world is the tomato (*Solanum lycopersicum*). Tomatoes are juicy and sweet (Valadez *et al*.2012, Agrios, 2005). The edible red berry on the shrub serves as its fruit. Despite being a fruit by definition, the tomato (*Solanum lycopersicum*) is categorized as a vegetable in commerce, according to Naika *et al*. (2005). It ranks among the most popular vegetables consumed globally (Chapagain and Wiesman, 2004). Originally from South America, tomatoes are a nightshade fruit (Adda, 2019). Due to its relatively short growing season and large yield, this crop is extremely important economically in many nations (Obeng-Ofori *et al.*, 2007) It is a crucial component of a healthy diet, a significant source of the antioxidant lycopene, and is packed with minerals. Customers have purchased it in large quantities because of its great nutritional content, deliciousness, accessibility, and affordability, which generates large profits for small-scale growers in poor nations (Mendus *et al.*, 2012). However, tomatoes are highly perishable and have a short shelf life due to their high-water content (95%), which makes them susceptible to spoiling by microorganisms, particularly pathogenic bacteria, when specific handling procedures are not followed. Additionally, its failure could seriously endanger customers' health (Muhammad *et al.*, 2011) Possibly harmful foodborne microorganisms could be present in tomatoes (Nutt *et al*., 2003). *Salmonella, Shigella*, E. *coli* 0157 H7, *Listeria, Campylobacter, Cryptosporidia*, and some viruses like Hepatitis A are some bacteria that are frequently linked to tomatoes (Anderson *et al*., 2011). In addition to the variety they bring to the table, these veggies are renowned for being excellent sources of nutrients. While only having about 35 calories, one medium tomato provides 40% of the Recommended Daily Allowance (RDA) of vitamin C (ascorbic acid), 20% of

the RDA of vitamin A, significant amounts of potassium, dietary fiber, and calcium, and lesser amounts of iron, magnesium, thiamine, riboflavin, and niacin (Tigist *et al*., 2013; Adekalu *et al*., 2016).According to the World Health Organization (WHO) consultation committee, a medicinal plant is any plant that contains compounds that can be used therapeutically or that serve as building blocks for the production of effective pharmaceuticals in one or more of its organs (Anie *et al.,* 2015; Ibezim *et al.,* 2011).In comparison to the other 39 major fruits and vegetables grown in Africa, tomatoes come in first place in terms of their relative contribution to human nutrition. The *Solanaceae* family includes the tomato (*Solanum lycopersicum*), which is frequently used both fresh and in the creation of various food products (Suri *et al*., 2017). Naturally occurring, non-pathogenic epiphytic bacteria are typically present in fresh produce. Nevertheless, hazardous viruses of human or animal origin frequently become a part of vegetables during their growth, harvest, transportation, and subsequent handling. The potential of microbial contamination to customers' health is very high because raw veggies are frequently consumed (Brandl, 2006).With the goals of determining the bacterial load on tomatoes sold in some central markets in Maiduguri and isolating and identifying bacteria from tomatoes sold in some central markets in Maiduguri metropolis, Borno State, Nigeria, this study was created to determine the pathogenic bacteria that are associated with tomatoes sold in some central markets in Maiduguri.

Materials and Method

Study Area

Three separate marketplaces (Tashan Bama, Custom, and Monday market) in Maiduguri were used as the subjects for an experimental study in which tomatoes were randomly chosen from various vendors. Because they are significant marketplaces in the area where tomatoes are sold to consumers, these markets were chosen.

Sample Collection and Samples Size.

From the three markets in Maiduguri, 30 samples of tomatoes in total were chosen at random. 5 fresh and 5 spoiled tomatoes were bought from each market. For bacteriological analysis, samples were immediately transported to the Department of Microbiology laboratory, faculty Science, University of Maiduguri, packaged separately into various sterile containers, labeled, and delivered. The time between

sample collection and analysis was no longer than two hours.

Laboratory Analysis

The approach used by Ugwu *et al*. (2014) to analyze tomatoes. Fresh tomatoes were quickly split into two equal halves after being thoroughly cleaned with sterile water (this was carried out because this is a common practice by consumers before food preparation). The interior of the tomatoes that had been sliced open was swabbed aseptically with a sterile swab stick. Before being incubated at 37°C for 18–24 hours, the swabs were streaked onto Nutrient agar, MacConkey agar, EBM, and SSA. For the spoiled tomatoes, samples were taken from the spoiled part of the tomatoes using a sterile swab. These samples were then streaked onto Nutrient agar, MacConkey agar, EBM, and SSA before being incubated at 37°C for 18–24 hours.

Bacteriological Analysis

A stock solution for serial dilution was created by homogenizing 10 grams (blended) of each sample into 90 milliliters of peptone water, putting one (1) milliliter into a test tube containing nine milliliters of sterile peptone water, and then serially diluting up to 10^5 times. Spread plate method technique was used to plate 0.1 ml of the suspension from the dilution of 10^3, 10^4, and 10^5 aseptically on a prepared Nutrient Agar (NA) and incubate at 300^C for 24 hours. Colony forming units (Cfu/g) of tomato were employed as a unit of measurement for the results, which were calculated by multiplying the counts by the dilution used. The isolates were also subculture in Nutrient agar, MacConkey agar, EBM, and SSA before being incubated at 37°C for 24 hours (Kiran *et al*., 2010).

Characterization of the Bacterial Isolates

Gram's Staining

Gram's staining was done in accordance with Harley and Prescott's instructions (2002). A drop of water was used to smear the bacterial isolate onto a glass slide that was clean and devoid of oil. The smear was passed over a flame to be fixed after being allowed to air dry. After fixing, a primary dye (such as crystal violet) was applied to the smear for one minute, and then it was washed with water. After one minute, Lugol's iodine was applied to the slide and it was then cleaned. The smear was quickly decolored with ethanol and then rinsed with water. The application of safranin was then made, which was then kept on for 30 seconds before being wiped off with water. A cotton wool pad was used to clean the slide's back, which was then

let to dry naturally. Utilizing the microscope's x100 oil immersion objective lens, the slides were inspected. Gram positive bacteria are typically bluish or purplish in color, while Gram negative bacteria are typically red or pink in color.

Catalase Test

On a glass slide with bacterial colonies added, a drop of 3% (v/v) H_2O_2 was applied. Oxygen bubble production revealed the presence of catalase (Harley and Prescott, 2002).

Triple Sugars Iron Test

Using a sterile transfer needle, the isolates were inoculated onto triple sugar iron slants. The butt was punctured with the needle, which was then removed after streaking the surface. The inoculated slants were then evaluated for gas production, hydrogen sulfide production, glucose, lactose, and sucrose fermentation after being incubated at 37^0C for 24 hours (Ochei and Kolhatkar, 2000).

Urease Production Test

In general bottles, streaking was used to inoculate slants of urea medium with a loopful of the isolates. These were observed every day while being incubated for 4 days at 37^0C. Urease positivity was shown by a change in color from pink to red (Ochei and Kolhatkar, 2000).

Methyl Red Reaction Test

A loopful of the isolates was added to a glucose phosphate medium that had been pre-prepared in a test tube, and it was then cultured at 37^0C for 4 days. Drops of methyl red solution were applied to the culture that had been active for four days. They were rubbed, then checked out. The methyl red reaction was successful, as evidenced by the surface of the reagent layer turning red (Ochei and Kolhatkar, 2000).

Voges-Proskauer Test

0.6ml of a 5% - napthol solution was added to the culture in the previous section and shaken. After 15 minutes, the test tubes were slanted and examined. A red coloration meant that the VP reaction was favorable (Ochei and Kolhatkar, 2000).

Indole Production Test

The isolate was injected in a loopful in a sterile nutritional broth. 48 hours were spent incubating at 37^0C. 0.5ml of Kovac's reagent was then added and shook following incubation. After a minute, this was evaluated. Indole synthesis was shown by the reagent layer's red coloring (Ochei and Kolhatkar, 2000).

Citrate Utilization Test

A loopful of the 24-hour-old isolate was aseptically added to a sterile Simon's citrate medium and cultured there for 24 hours at 37^0C. Over the course of three days, the medium's turbidity was checked each day. Turbidity indicated the use of citrate (Ochei and Kolhatkar, 2000).

Results

The results for the tomatoes' ph. and moisture content from the sampling locations Tashan bama, Custom market, and Monday market are displayed in Tables 4.1a, 4.1b, and 4.1c. The pH of the tomatoes from all the sampling locations ranges from 3.30 to 4.60, which is a little bit the same. When fresh tomatoes were tested at Tashan Bama, the pH value was 4.60, but spoiled tomatoes were tested at Custom Market and yielded a pH value of 3.30. The varied tomatoes in the samples' moisture content range from 95.2% to 93.6%. The fresh tomato had the highest moisture content, whereas the spoiled tomato had the lowest (Table 4.1a. b, c).

Table 4.1a: Average Values of pH and Moisture Content Obtained from the Tomato Samples of Tashan Bama

Sample code	pH Value	Moisture Content (%)
TBF1	4.60	95.0
TBS1	4.34	95.1
TBF2	4.41	94.0
TBS2	4.45	95.2
TBF3	3.35	94.1
TBS3	4.42	95.0
TBF4	4.53	95.2
TBS4	4.50	95.0
TBF5	4.47	95.1
TBS5	3.45	94.5

Key: TBF: Tashan Bama Fresh, TBS: Tashan Bama Spoilt

Table 4.1b: Average Values of pH and Moisture Content Obtained from the Tomato Samples of Custom Market

Sample code	pH Value	Moisture Content (%)
CUF1	4.40	93.6
CUS1	4.34	95.1
CUF2	4.41	94.0
CUS2	3.45	95.2
CUF3	4.40	94.1
CUS3	4.42	95.0
CUF4	4.40	95.2
CUS4	3.30	94.0
CUF5	3.47	95.1
CUS5	4.45	94.0

Key: CUF: Custom Market Fresh, CUS: Custom Market Spoilt

Table 4.1c: Average Values of pH and Moisture Content Obtained from the Tomato Samples of Monday Market

Sample code	pH Value	Moisture Content (%)
MMF1	4.25	95.0
MMS1	3.34	94.1
MMF2	4.41	94.5
MMS2	3.45	93.7
MMF3	4.40	94.1
MMS3	3.62	95.0
MMF4	4.53	95.2
MMS4	4.50	94.0
MMF5	4.47	94.1
MMS5	3.45	93.0

Key: MMF: Monday Market Fresh, MMS: Monday Market Spoilt

Tables 4.2a, 4.2b, and 4.2c show the numbers of viable aerobic bacteria. Between $4.80x10^6$cfu/g and $1.38x10^6$cfu/g of bacteria per gram of tomato were found in the sampling locations Tashan bama, Custom market, and Monday market. The spoiled tomato in Tashan Bama had the greatest bacterial level, $4.80x10^6$cfu/g, while the fresh tomato at Monday market site had the lowest bacterial count, $1.38x10^6$cfu/g.

Table 4.2a: Total Aerobic Count Obtained from the Tomato Samples of Tashan Bama Site

Sample code	Bactierial colony count (cfu/g)
CUF1	2.45×10^6
CUS1	1.34×10^6
CUF2	3.45×10^6
CUS2	1.86×10^7
CUF3	2.23×10^6
CUS3	$3.56x\ 10^6$
CUF4	2.89×10^6
CUS4	$2.57x\ 10^6$
CUF5	2.48×10^6
CUS5	$4.80x\ 10^6$

Key: CUF: Custom Market Fresh, CUS: Custom Market Spoilt

Table 4.2b: Total Aerobic Count Obtained from the Tomato Samples of Custom Market Site

Sample code	Bactierial colony count (cfu/g)
CUF1	1.51×10^6
CUS1	3.34×10^6
CUF2	1.45×10^7
CUS2	2.86×10^7
CUF3	2.23×10^6
CUS3	3.56×10^6
CUF4	2.89×10^6
CUS4	3.57×10^6
CUF5	1.48×10^6
CUS5	2.70×10^6

Key: CUF: Custom Market Fresh, CUS: Custom Market Spoilt

Table 4.2c: Total Aerobic Count Obtained from the Tomato Samples of Custom Market Site

Sample code	Bactierial colony count (cfu/g)
CUF1	1.51×10^6
CUS1	3.34×10^6
CUF2	1.45×10^7
CUS2	2.86×10^7
CUF3	2.23×10^6
CUS3	$3.56x\ 10^6$
CUF4	2.89×10^6
CUS4	$3.57x\ 10^6$
CUF5	1.38×10^6
CUS5	$2.70x\ 10^6$

Key: CUF: Custom Market Fresh, CUS: Custom Market Spoilt

Table 4.3 presents the findings about the morphological and biochemical properties of the bacterial isolates. At the sampling locations Tashan bama, Custom market, and Monday market, a total of twenty (20) bacterial isolates from the tomatoes were found.Escherichia coli, Enterobacter spp., and Klebsiella spp. are the three (3) identified bacterial genera (Table 4.4). Escherichia coli was determined to be the most prevalent specie among the isolates and had the greatest percentage frequency of occurrence (50%) of any isolate. followed by Enterobacter spp (30%), while Klebsiella spp. had the lowest incidence rate (20%). (Table 4.3).

Table 4.3: Morphological and Biochemical Characteristics of the Bacterial Isolates

Code	Shape	Gra	Cat	MR`	Ure	Ind	Cit	Lac	VP	Organism
TBF1	Rod	-	+	+	-	+	-	+	-	*E. coli*
TBS2	Rod	-	+	+	-	+	-	+	-	E. *coli*
TBF3	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
TBS4	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
TBF2	Rod	-	+	+	-	+	-	+	-	*E. coli*
TBS3	Rod	-	+	-	-	-	-	-	+	*Enterobacter sp*
TBF5	Rod	-	+	-	-	-	-	-	+	*Enterobacter sp*
TBS5	Rod	-	+	-	-	-	-	-	+	*Enterobacter sp*
TBS2	Rod	-	+	+	-	+	-	+	-	*E. coli*
CUF1	Rod	-	+	+	-	+	-	+	-	*E. coli*
CUS1	Rod	-	+	+	-	+	-	+	-	*E. coli*
CUF1	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
CUF2	Rod	-	+	+	-	+	-	-	-	*E. coli*
CUS4	Rod	-	+	+	-	+	-	+	-	*E. coli*
CUF5	Rod	-	+	+	-	+	-	-	-	*E. coli*
CUF2	Rod	-	+	+	-	-	-	-	+	*Enterobacter sp*
CUS4	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
MMF1	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
MMS2	Rod	-	+	+	-	-	-	-	-	*Klebsiella* sp.
MMF4	Rod	-	+	+	-	+	-	+	-	E. *coli*

Key: Gra-Gram Reaction, Cat-Catalase, MR-Methyl Red Reaction, Ind-Indole, Cit-Citrase, Glu-glucose, Lac-Lactose, VP- Voges-Prosk

Table 4.4: Frequency of Occurrence of Identified Bacterial Isolates from the Tomato Samples

S/No.	Identified Isolates	Number of Occurrence	Percentage Frequency of Occurrence (%)
1	*Escherichia coli*	10	50
2	*Enterobacter* spp	6	30
3	*Klebsiella* spp.	4	20
	TOTAL	20	100

Discussion

Since fresh vegetables have become new vectors for the spread of various infectious diseases, the epidemiology of food-borne disease has changed drastically over the years. Fresh vegetable consumption has been linked to recorded outbreaks of disease progressively since the early 1990s, as has public awareness of the possibility that fresh produce could spread certain food-borne illnesses (Falomir *et al*., 2010). The pH of the sample determines how acidic, alkaline, or neutral the tomato is, which has an impact on how long bacteria can survive. This study's pH values, which ranged from 3.30 to 4.60 and were acidic, were comparable to those of Ogundipe *et al*. (2012), who investigated the prevalence of bacteria with potential public health implications in raw *Lycopersicon esculentum* (tomato) sold in Lagos State, Nigeria. Furthermore, this outcome was consistent with Javaria's (2012) analysis of the impact of potassium on the chemical and sensory characteristics of tomato fruit. A tomato can be consumed uncooked and is a very perishable fruit or vegetable. High solute content and water activity in this area promote microbial development (Uria and Izuaghe, 1990; Charley, 2002). According to Sohail *et al*. (2011), fresh tomatoes had a moisture content of 94.4 percent on average, which the study's average moisture content corresponds with (Adebanwo *et al*., 2002). It is well recognized that an unorganized food structure and weakened or broken natural barriers to protection increase the risk of microbial contamination. The tomato samples'

total aerobic bacterial counts ranged from $1.38x10^6$cfu/g to $4.80x10^6$cfu/g (Table 4.2abc). The results of the present investigation are in agreement with those of Ogundipe *et al*. (2012), who investigated the prevalence of bacteria with potential public health implications in raw *Lycopersicon esculentum* (tomato) sold in Lagos State, Nigeria. Bacterial contamination, particularly with members of the Enterobacteriaceae family, has been connected to the majority of recorded outbreaks of gastrointestinal sickness linked to fresh vegetables (DuPont, 2007). Additionally, the existence of antibiotic resistance in normal flora and pathogenic microbes in fresh vegetables may contribute to the horizontal transmission of resistance between various isolates, species, and genera (De La Cruz and Davies, 2000; Tenovar, 2006; Heuer and Smalla, 2007).The outcome of this investigation is comparable to that of Ogundipe *et al*. (2012), who investigated the incidence of bacteria with potential public health implications in raw Lycopersicon esculentum (Tomato) sold in Lagos State, Nigeria.

Conclusion

The findings of this study highlight the significant diversity of bacterial isolates associated with tomato fruits, underscoring their potential role as vehicles for the transmission of pathogenic microorganisms to consumers. The presence of such bacteria raises important public health concerns, as contaminated tomatoes may serve as sources of food poisoning, gastrointestinal infections, and other food-borne illnesses. Considering that fresh produce, including tomatoes, is often consumed raw or with minimal processing, the risk of disease transmission is considerably higher compared to thermally processed foods. Moreover, because fresh tomatoes are widely distributed across markets and households, outbreaks linked to contaminated produce can rapidly spread within and between communities, resulting in large-scale epidemics. This risk is further heightened when fruits are bruised, damaged, or improperly handled, as such conditions can promote microbial growth and facilitate pathogen invasion into the fruit tissue. So Therefore, it is imperative that preventive measures be adopted at every stage of the food chain—from farm production and harvesting to transportation, storage, and final consumption. More so, the results of this study emphasize the need for strict hygiene practices and proper handling of tomatoes to safeguard public health.

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