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Occurrences of Bacterial Isolates Associated with Tomato Rot in Two Markets of Jos Metropolis, Nigeria

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Abstract:
Study was conducted to determine the occurrences of some bacteria isolates associated with tomato rot in two markets of Jos metropolis. Rotten ripe tomato fruits were aseptically collected from Terminus and Farin-gada markets of Jos metropolis and Pour plate cultural method was used to isolate the bacterial species and the microbial load in a given sample from each market. Results of macroscopic examination of the spoiled tomato samples collected from the two major tomato markets (Terminus and Faringada markets) within Jos metropolis showed that some of the tomatoes had lost their fitness leading to the loss of usual shape, some had a black, whitish spot and broken epicarps where their juices and seeds gushes out when compressed. It had slightly unpleasant smell. The result of the isolation of bacteria from the spoilt tomatoes fruit showed the presence of seven bacteria (Klebsiella sp., Pseudomonas sp., Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Erwina sp. and Shigella sp.) isolates. The percentage frequency of occurrences of the bacterial isolates in the two markets showed that Klebsiella sp. had the highest occurrence of 28 (22%) while Staphylococcus aureus had the lowest at 7 (5%). The result of the bacterial load of the spoiled tomatoes between the two markets using Nutrient agar and MacConkey agar CFU count showed that the bacterial load from Terminus market was higher than Farin-gada market. Meanwhile, result of the comparison of the frequency of occurrences of bacterial isolates in relation to the organisms showed a high significant difference ($P<0.05$) from both markets. However, there was no significant difference ($P>0.05$) in the frequency of occurrences of the bacterial isolates in relation to the markets. The bacterial count recorded for the two markets indicated a high level of spoilage bacterial contamination of the tomato fruits. Thus, possible health risks may be associated with the consumption of relatively cheaper but spoilt ripe tomato fruits as well, an agent of food borne bacterial diseases

Keywords: *Occurrence, Bacteria isolates, tomato rot, Jos*

1. Introduction

Tomatoes (*Lycopersicon esculentum* L.) belong to the family Solanacea (night shade family). This species produces elongated or heart-shape (5 inch in diameter) tomatoes fruits on grape like clusters on long, trailing vines, they also have a distinctive, less acidic flavor and watery texture, not withstanding, quite decisions. They require staking, trellising or caging and their fruits usually are lighter than the determinate fruit types. They grow continuously and produce fruit as long as conditions are favorable. Wild type tomatoes species are said to originate from Western South Africa and especially in the coastal desert of Peru. First historical reference in Europe to tomatoes was a yellow-fruited type in Italy in 1544 and cultivated in Germany in 1553 (Chambers, 1979). The Italians were said to have acquired it from Turks. Tomatoes was first grown in Spain, and then taken to Italy where it was called "pomd" "oro" or golden apple, because of its yellow colour. There are over a hundred varieties of available tomatoes than other vegetables among which includes; *Lycopersicon esculentum*, *Lycopersicon lycopersicum* and *Lycopersicon hirsutum*. Most tomatoes are normal but some appear in pear shapes, elongated, flattened and heart shape. Tomatoes vary in their acid composition, with white and yellow ones being less acidic. Tomatoes varieties can be either determinate or in-determinate. Determinate tomatoes plants are small and spinally have just one fruiting or vent. The indeterminate types continue to grow and produce fruit as long as conditions are favourable. They require staking a caging and their fruit usually is higher in sugar than determinate types.

Tomatoes are among the vegetable products cultivated worldwide that has the greatest nutritional value (Thompson, 1998). World tomato production in 2001 was about 105 million tons of fresh fruit from an estimated 3.9 million hectares (FAO, 2005). In Nigeria, the pear shape orange colour tomato accounts for about 18% of the average daily consumption of vegetables (Babalola*et al.* 2010);

this makes it a very important food crop to an average Nigerian. With about 1,107,000 tons of tomato produced in 2008, making Nigeria become the 13th largest producer of tomato in the world that year (FAO, 2010).Tomato contributes to a well- balanced diet been rich in vitamins A, B, C and D (USDA, 2010), with its carbohydrate content including: fructose and glucose, it has also been shown to contain minerals such as phosphorus, sodium, potassium, calcium, magnesium and trace elements like iron, copper, zinc and dietary fibers (Rao,2007; USDA, 2010).The deep red colouration of ripened tomato fruit has been attributed to the presence of leucopenia, a form of β- carotenoid pigment, a powerful antioxidant that help to protect against prostate cancer, cardiovascular diseases and diabetes (Basu and Imrhan, 2006) Tomato fruit is a common vegetable eaten raw as salad or for garnishing various cooked food in Nigeria as well as in many parts of the world and are among the vegetable products cultivated worldwide with great nutritional value (Thompson, 1998). The skin of tomato fruits is a good form of lycopine, a substance that has been shown to protect people from heart attacks (Anon, 2001). Tomato is rich in vitamin C and other essential body nutrients. Lycopine has also been shown to have a very beautiful effect upon the prostrate and is being used increasingly to treat prostrate and difficulties in urination that accompany this disorder (Huxley, 1992).

Tomatoes are said to be spoiled, when their characteristic are changed so that it is no longer good for consumption (Ugwu*et al.*, 2014). To spoil is to deprive of good or effective qualities. Such changed may not always be micro biological in origin, a product may become unacceptable as a result of insect damage, drying out, discoloration or stating for instance, but by and large most food spoilage is as a result of microbial activity (Aworth, 1985; Uzeh *et al.*,2009). A general feature of microbial spoilage is its related sudden onset. It does not appear to develop gradually, day by day a little worse, but more often as an unexpected and unpleasant revelation (Ugwu*et al.*, 2014). This is a reflection of the exponential nature of microbial growth and its consequence that microbial metabolism can also proceed at an exponentially increasing rate (Brackett, 1992).

Despite its importance, tomato fruits have been faced with diseases causing rots which lead to loss of quality and also substantial postharvest loss(Aworth, 1985; Uzeh *et al.*,2009). There are many disease pests of tomato including bacteria, fungi and many viruses. The bacterial group (*Pseudomonas solana ceapum*) is a soil borne bacterium which infects the root and stem of the plant causing sudden disease. Crop rotation and sanitation are control measures for this. Bacterial canker, (*Corynebacterium michiganerise*) is a seed borne bacterium is which cankers form on the sterile and petioles light halos may form on the berry. The use of disease free seed is the best control from canker. Bacterial speck (*Pseudomonas singe*) is a problem in most weather. Dark green halos on fruit characterize this disease (Chukwuka, 2013).Removing dead and decaying plant matter and other organic material from the crop plant and soil surface will eliminate a major harborage for spoilage microbes as well as other crop pests. During harvest, transportation and storage the biological structure of tomato fruit could be disrupted serving as a route of entry for opportunistic pathogens due to their soft textured fruits (Ugwu*et al.*, 2014). Bukar*et al.* (2010) states that due to poor storage condition, resistance of fruit and vegetables to natural diseases usually decline, leading to infection by pathogen. Predominantly, these bacteria results in changes in the condition of the fruit. High water level of tomato fruit provides a conducive environment for these pathogens; reducing its palatability with increased toxicity levels(Aworth, 1985; Uzeh *et al.*,2009). These changes may be accompanied by alteration in its tastes, smell, appearance or texture (Ugwu*et al.*, 2010). This phenomenon usually referred to as spoilage in tomato. Tomato spoilage would therefore be simply defined as those adverse changes in quality of tomatoes, which are brought about by action of predominantly biological factors and physical factors (Ugwu *et al.*, 2010). Due to poor post-harvest handling and because they are seasonal crops; this has made their microbiological study necessary so as to identify their spoilage organisms (Uzeh *et al.*,2009). Research efforts over the years have helped in the increase in production of tomato but the purpose of obtaining maximum profit will be served only if the increased production is supplemented with the similar efforts to minimize the postharvest losses and enhance shelf life. This has made their microbiological study necessary so as to identify their spoilage organisms so as to find suitable means to minimize post-harvest losses (Frederick, 1983, Nasrin*et al.*,2008). In recent years, the incidence of spoilage in tomato fruits has been a cause for global concern, hence intensive research has been in progress to comprehend the measures which can be taken to control this menace. A close study on the intended work could bring an answer to this global concern. The specific aim of this research is to isolate and determine bacterial loads and the percentage occurrences of bacteria causing spoilage in tomato fruit rot and to make a comparative study on the bacterial loads between Terminus and Farin-gadamarkets in Jos metropolis.

2. Materials and Methods

About 200 spoilt tomatoes each were picked up respectively from two selected market of Jos; namely Farin-gada and Terminus and placed in separate sterile plastic bags transported to the pharmacognosy laboratory in the University of Jos for bacterial analysis.

2.1. Isolation of Bacteria

Pour plate cultural method was used to isolate the bacterial species. The spoilt tomatoes were washed in sterile water to reduce the microbial load after which sterile forceps were used to pull apart the tomatoes at the site of spoilage, 1g of the monocarp from the advancing margin of the scar were each aseptically transferred into 10ml of sterile normal saline into various beakers according to the number of samples collected. The tomatoes fruits in their respective beakers were shaken to dislodge the organisms and allowed to stand in the normal saline for 10 minutes, after which they were removed from the beakers with the aid of sterile forceps.

Six text tubes containing 9ml of sterile normal saline each were placed on a rack on the bench, using test tube rack. 1ml from the sample solution was pipette aseptically into the first test tube and mixed. It was then transferred to the second test tube and mixed and repeated up to the last tube (10^{-6}). Then 1ml from the last test tube was discarded after which 1ml of each of the dilution was plate out on nutrient agar (pH 5.5) and MacConkey agar. The plates were incubated aerobically at 37^0C for 24 hours.

2.2. Enumeration of Bacteria

Microbial count was carried using the method by Onyeagba, (2004) to determine the microbial load in a given sample from each market. This was done by counting the bacterial colonies that appeared on the agar plates. Bacterial count was determined by multiplying the number of colonies by the volume of inoculums and the dilution factor.

2.3. Preparation of Pure Culture

To obtained pure bacterial cultures, distinct colonies were aseptically transferred into newly prepared sterile Nutrient Agar by streaking, using sterile wire loop. The pure isolates were later transferred into sterile Agar slants in Bijou bottles. Samples for identification tests were taken from the agar slants.

2.4. Characterization and Identification of Isolates

Bacterial Isolates were characterized and identified by macroscopic and microscopic examinations, and biochemical tests. The biochemical tests included: indole test, catalose test, motility test, citrate utilization test, oxidase test, starch hydrolysis test and sugar fermentation tests (Cheesbrough, 2006).

2.5. Data Statistical Analysis

Data was analyzed using R Console version 3.2.2. Student t-test was used to compare the mean colony forming unit in tomatoes between the two sites. Proportions of occurrence for each organism in relation to collection sites were compared using Pearson's Chi-square test. Also, chi square test was used to compare the proportion of occurrence across organisms. The P-values < 0.05 were considered statistically significant.

3. Results

Macroscopic examination of individual spoiled tomato samples collected from the two major tomato market sites (Terminus and Faringada markets) within Jos metropolis were identified based on their cultural, morphological, microscopic and biochemical identification and characterization as presented on Table 1. The fruits had lost their fitness leading to the loss of its usual shape, brokenblack spots at epicarp and a slightly unpleasant smell.The bacterial isolates from the spoiled tomato fruit samples the bacterial isolates identified were; *Klebsiella* sp., *Pseudomonas* sp., *Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Erwina* sp. and *shigella* sp.

ORGANISM IDDENTIFIED	**MACROSCOPIC EXAMINATION**		**MICROSCOPIC (Gram Stain)**		**BIOCHEMICAL IDDENTIFICATION AND CHARACTERIZATION**													**Frequency of occurrence / percentage**	
	OPACITY	COLOUR	Shape	Gram rxn	Catalase	Coagulase	Indole	Urease	Citrate	Oxidase	Motility	Glucose	Sucrose	Lactose	Slope	Butt	Gass	H_2S	
Klebsiellasp	Opaque	Creamy white	Circular	-	+	+	-	+	+	-	+	+	-	-	Y	Y	-	-	28 (22%)
Shigellasp	Transparent	Pale	ROD	-	+	ND	-	-	-	-	-	+	-	-	R	Y	-	-	25 (19%)
Erwinasp	Opaque	Milky	ROD	-	+	ND	+		-	-	+	+		-	Y	Y	+	+	22 (17%)
Salmonella sp	Transparent	CREAMY WHITE	ROD	-	+	+	-	+	+	-	+	+	-	-	R	Y	-	+	19 (15%)
Proteus sp	Transparent	Transparent	ROD	-	+	+	-	+	+	-	+	+	-	-	R	Y	-	-	16(12 %)
Pseudomonas sp	Transparent	Creamy light green	ROD	-	+	+	+	+	+	+	+	+	+	+	R	R	-	-	12 (9%)
Staphlococcus aureus	OPAQUE	GOLDEN YELLOW	COCCI	+	+	+	+	-	+	-	+	+	+	+	R	Y	-	-	7(5%)

Table 1: Macroscopic, Microscopic and Biochemical Identification and Characterization of Isolates with Their Individual Frequency of Occurrence

The result of the bacterial count of the spoilt tomato samples using two media (Nutrient and MacConkey agar) from each market revealed that the mean bacteria count of spoiled tomato sold in Terminus market of Jos metropolis had a higher mean count than that from Farin-gada market. However, statistically, there was no significant difference (P>0.05) between bacterial loads of the two markets as presented in Tables 2 and 3.

MEDIA	DILUTIONS	CFU Count for Markets	
		Terminus	Farin-gada
NUTRIENT AGAR	10^{-1}	1.6×10^3	1.1×10^3
	10^{-2}	1.0×10^4	7.8×10^4
	10^{-3}	6.7×10^4	5.3×10^4
	10^{-4}	4.2×10^5	3.4×10^5
	10^{-5}	2.8×10^6	2.5×10^6
	10^{-6}	1.5×10^7	1.3×10^7
MEAN ± S.E		**3049767 ± 2430463**	**2662017± 2104412**

Table 2: Bacterial Count from the Terminus and Farin-Gada Market Using Nutrient Agar (N.A)

MEDIA	DILUTIONS	CFU Count for Markets	
		Terminus	Faringada
MACCONKEY AGAR	10-1	1.0×10^3	8.7×10^2
	10-2	6.0×10^3	6.6×10^3
	10-3	4.6×10^4	4.0×10^4
	10-4	3.4×10^5	2.5×10^5
	10-5	1.8×10^6	1.4×10^6
	10-6	9.0×10^6	6.0×10^6
MEAN ± S. E		**1865500 ± 1454615.8**	**1282912± 968658.4**

Table 3: Bacterial count from the Terminus and Farin-gada market using MacConkey Agar (MCA)

3.1. Results of Comparison of the Frequency of Occurrence between Sites for Each Organism

There was no significant difference in the frequency of occurrence of all the bacterial organisms isolated between all the sites (Table 4).

The bacterial isolates identified were: *Klebsiella* sp., *Pseudomonas* sp., *Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Erwinas* sp. and *shigella* sp. Meanwhile, *Klebsiella* sp. had the highest occurrence at 28 (22%), followed by *Salmonella typhi* 25 (19%) then *Shigella* sp.22 (17%) and *S. aureus* had the lowest occurrence of 7 (5%). However, statistically there was a significant difference (P<0.05) in the frequency of occurrence in relation to organisms from both markets and no significant difference (P>0.05) in the frequency of occurrence between sites for each organism (Table 4).

Organism	Total no of plates	No. of Occurrences for each organism on		+ve no of plates from	-ve no of plates from			
				Terminus	Faringada	d_f	X^2	P_{value}
		+ve	-ve					
*Klebsiella*sp.	129	28	101	16	12	1	0.571	0.4497
Salmonella typhi.	129	25	104	14	11	1	0.360	0.5485
*Shigella*sp.	129	22	107	15	7	1	2.909	0.088
*Erwina*sp.	129	19	110	8	11	1	0.473	0.491
Proteus mirabilis.	129	16	113	10	6	1	1.000	0.317
Pseudomona sp.	129	12	117	7	5	1	0.333	0.563
Staphlococcus aureus	129	7	122	3	4	1	0.143	0.706

Table 4: Occurrences of Individual Organisms in Terminus and Faringada Markets

4. Discussion

Fresh fruits have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms. However, this protection may be eliminated and fruits may become contaminated during their growth in fields or during harvesting; post-harvest handling and distribution from where these spoilage organisms get access into the fruits and cause spoilage (Bukar *et al.* 2010). Such changes may not always be micro biological in origin, a product may become unacceptable as a result of insect damage, drying out, discoloration or stating for instance, but by and large most food spoilage is as a result of microbial activity (Wogu *et al.*, 2014); which is our primary concern in this context. A general feature of microbial spoilage is its related sudden onset. It does not appear to develop gradually, day by day a little worse, but more often as an unexpected and unpleasant revelation. This is a reflection

of the exponential nature of microbial growth and its consequence that microbial metabolism can also proceed at on exponentially increasing rate (Brackett, 1992).Fruits and vegetables do have a high-water activity but they develop another spoilage scenario than meat, fish and milk. Many of these products are protected mechanically by the pectins which constitute a "glue" between the cells and gives rigidity. When fruits and berries ripen, endogeneous pectinases start to hydrolyse the pectin and this also makes the products more susceptible to microbial attacks. The primary causative agents of microbial post-harvest spoilage of tomatoes are the bacteria, yeasts and moulds (Aworth, 1985; Uzeh *et al.,* (2009). Due to the high proliferation rate of bacteria, they are considered highly as a more frequent cause of spoilage of tomato (Abadias *et al.,* 2008). Work by Villareal, (1980) showed that wounded tomato fruits were most liable to microbial infection and hence deterioration. A cut on a broken tomato fruit may easily harbour pathogens that may spread and spoil all tomatoes in a lot (Wogu *et al.,* 2014). The bacterial count recorded for the two-market site indicated a high level of spoilage bacterial contamination of the tomato fruits. The isolation of these spoilage bacteria due to contamination on the tomato samples was evident of opportunistic contamination from mostly human activity Wogu,(2014). The mean bacterial counts in the spoiled tomato fruits samples investigated in this study were similar to those obtained in other studies in Nigeria by Uzeh*et al.*,(2009); Bukar*et al.*,(2010). The high microbial contamination observed in the fruits in this study may also be a reflection of storage conditions and how long these produces were kept in the open or in stores before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water by the vendor or processor. More importantly, bacteria on the produce may multiply over time depending on the storage conditions especially those that are psychotropic (Montville and Matthews, 2008; Abadias *et al.* 2008). Considering the notoriety of the resistance of *S. aureus* to methicillin, other penicillin and celphalosporins (Adeleke and Odelola, 1997), its detection in tomato samples poses a lot of health risk to nourishment seeking consumers even though it's occurrence in this present study within the two major markets of Jos metropolis was very low. Also, the high bacteria count could arise from the fact that tomato sellers leave these fruits in the open under the sun where they are heated up and subjected to rotting due to the heat. Matthew, (2011), has proved that temperature has an effect and in fact high, temperatures encourage deterioration of tomato fruits, and that high temperature levels speed up the physiologic processes occurring in fruits leading to sub-oxidation and accumulation. Full ripe tomatoes can be stored at a temperature of 55^0f for up to several days. Temperature cooler than this will cause chilling injury producing poor colour and off flavours (Huxley, 1992).

This present study had revealed the present of seven (7) bacterial isolates:(*Klebsiella*sp., *Shigella*sp., *Erwina*sp., *Salmonella* sp., *Proteus* sp., *Pseudomonas* sp. And *Staphlococcusaureus*) whichhadbeen previously isolated from rotten tomato fruits in other studies; both in Nigeria and other parts of the world (Uzeh *et al.* 2009; Ashok *et al.* 2011; Angela *et al.*2010).However, this work is contrary to the previous work of (Wogu *et al.,* 2014); Mathew, (2011) who allisolated *Bacillus, Listeria, Morgnella, Xanthomonas* and *Lactic acid* bacteria from the spoilt tomatoes. This could be as a result of varietal characteristics, like harvesting methods; sometimes mechanized and sometimes hands picking is used, climate influence, geographical and seasonal variations and also various internal and external sources of contamination and agro-technical procedures which include the use of contaminated irrigation water, use of animal and human wastes as fertilizers, improper handling and storage (Effurvwevwere *et al.*, 2000). For example, tomatoes for the fresh market are generally handpicked; Processing tomatoes are picked fully ripe. Some of the bacteria isolated in this study may be part of the natural flora of tomato or contaminants from soil, irrigation water, and the environment during transportation, washing/rinsing water or handling by processors (Ofor*et al*., 2009). *Pseudomonas* sp. is part of the natural flora and is among the most common vegetable spoilage bacteria as well as *Erwina*sp. which belongs to the family Enterobacteriaceae; are all associated with plants where they are known to cause plant diseases of the rot and wilt type. These gram-negative rods that are related to the genera *Proteus, Serratia, Escherichia, Salmonella* and others and can be indicators of feacal contamination.

5. Conclusion

Fruits and vegetables are very important and have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other importance cannot be over emphasized, as its spoilage often result to wastage of economic resources as well as food poisoning. From the results obtained in this study, some genera of bacteria have been identified in associated with the spoilage of tomato fruits and majority are food borne pathogens. It is also revealed that some spoilage microorganism gained access into these fruits during the processes of cultivating, harvesting, grading and packing and environmental contaminant which have in one time or the other been involved in food poisoning. The prevalence frequency of occurrences of *Klebsiella* sp., followed by *Salmonella* sp. and *Shigella* sp. were higher than that of other bacteria in this study are host specific organisms that have been contaminated with water containing human feces and grow only in the intestines of humans and apes. Thus, food borne infections are most likely to occur. The probably most common microbial food intoxication is caused by certain strains of *Staphylococcus aureus.* This organism is also known as a common pathogen causing infections in wounds and blood, but these infections are not considered to be transferred via food. *Staphylococcus aureus* produces a series of toxins and other virulence factors but it is mainly the enterotoxins that cause food poisoning after ingestion of food on which *S. aureus* has grown and produced the enterotoxins. *Salmonella* is environmentally very resistant which explains why they are widely spread in nature even if they grow mainly in animals. It is also common in spices and vegetables, probably through contamination with infected water or soil. The disease breaks out after 12-48 hours and lasts for a couple of days, with some exceptions when there are complications with reactive arthritis or septicemia with subsequent infection of organ systems and some strains of the pathogen can cause severe illness if not check. *Shigella* contrary to *Salmonella* and *Klebsiella* organisms are very host specific and grow only in the intestines of humans and apes. *Shigella* multiplies intracellular in the epithelial cells which results in tissue destruction. The high prevalence of bacteria on this fruit which leads to the fruits spoilage and the fact that they are still up for sale in local markets demand that appropriate control measures against infection should be employed to curtail loss

of commodities and human lives. It is therefore, important that both the farmers who harvest the fruits into bags for transportation, the marketers, and consumers take necessary precautions in preventing contamination and eating contaminated fruits. This will however, enhance reduction in the risk of microbial toxins that are deleterious to human health which are produced form these microorganism that have been isolated.

6. Recommendation

Based on the results gained from this study, it is recommended that concerted efforts be made by the relevant health workers to discourage or stop the display and sale of spoilt tomato fruits in local markets. Furthermore, the future prospects of the current research would include, further evaluations of plant remedies with the pure compounds for the definite conclusion of the bioactive compounds contributing to antimicrobial activity. This finding can form the basis for further studies to prepare an adequate microbiological knowledge and handling practices of tomato fruits which will help minimize wastages due to deterioration.

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