GRAM NEGATIVE BACTERIA ASSOCIATED WITH SPOILAGE OF TOMATOES SOLD AT MARARABA AND MASAKA MARKET, NEW KARU LOCAL GOVERNMENT AREA, NASARAWA STATE.

Olokun A.L[1*], Ajide A.B[1], and Ihuma J.O[1]
lanzyko07@yahoo.com

Department of Biological Sciences, Bingham University New Karu, Nasarawa State

ABSTRACT

Aims: The study is aimed at isolating and identifying the gram negative bacteria associated with spoilage of tomatoes at Mararaba and Masaka markets, New Karu Local Government Area, Nasarawa State.
Study design: A cross-sectional study to isolate gram negative bacteria associated with spoilage of tomatoes at Mararaba and Masaka markets, New Karu Local Government Area, Nasarawa State.
Place and duration of study: The isolation of gram negative bacteria was carried out at the Department of Biological Sciences, Bingham University New Karu, Nasarawa State, Nigeria, between January, 2018 and September, 2018.
Methodology: The samples collected were crushed with a sterile mortar and pestle after which tenfold serial dilution was carried out using sterile distilled water before inoculating on MaCconkey and Blood agar using the pour plate technique. Colonies were then subjected to gram staining and biochemical test.
Results: From the result of the biochemical test, *Salmonella* spp*, Esherichia coli* spp*, Klebsiella* spp *and vibrio* spp*.* were the gram-negative bacteria isolated from the tomato samples collected at both markets.
Conclusion: Tomatoes sold in an unclean environment that is contamination with food- borne pathogenic bacteria poses a risk to the consumers.

Keywords: Tomato, Market, Salmonella, Escherichia coli, Klebsiella and Vibrio spp.

1.0 INTRODUCTION

Tomato is scientifically known as *Solanum lycopersicum*, it's a berry plant in the *solanaceae* family and an edible often red coloured berry fruit [1]. It is a perishable perennial plant, grown as an annual crop, typically grown to about 3-5m approximately in height. [2].

Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well-balanced diet that is rich in vitamins such as A, B, C and E; Carbohydrates content such as fructose and glucose; Minerals which include phosphorous, sodium, potassium, calcium, magnesium and trace elements like iron, copper, Zinc and Dietary fibers [3]. Tomato is widely used as condiment or as food dietary supplement in various part of the world. It is also valuable in the food industry [4].

Spoilage refers to any change in the condition of food in which the food becomes less palatable or even toxic and these changes may be accompanied by alteration in taste, smell, appearance or texture[5]. Susceptibility of tomato to microbial colonization is due to its differential chemical composition such as high level of sugar, low pH (4.9-6.5) and its water activity (p>0.99) which favours the growth of microorganisms in tomato, hence, a source of potential health hazard to man due to production of toxins which are capable of causing diseases like gastroenteritis, diarrhoea in man following ingestion [6].

The vulnerability of tomato to contamination by mostly *salmonella* and other major human pathogens that are common cause of food-borne illness has remained a mystery. If the illness is not detected early and controlled, it can cause death [7]. According to [8], vegetables and fruits have been associated with outbreaks of food-borne diseases in many countries. Interestingly, pathogenic Gram-negative bacteria account for approximately 69% of the cases of bacterial food-borne disease [9].

The contamination of tomato by micro-organism could be as a result of poor handling practices in the tomato production chain; harvesting, storage condition, distribution, marketing practices and transportation [10].Tomatoes was chosen for this study because its referred to as ready to eat food, minimally processed and a lot of people take tomatoes raw or via meals of salad.The aim of this study was to isolate and identify the gram negative bacteria associated with spoilage of tomatoes at Mararaba and Masaka Markets, New Karu Local Government Area, Nasarawa State.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Fresh Samples of spoiled tomatoes were collected from two major markets within Nasarawa metropolis; which are Masaka and Mararaba market. A total of 100 tomatoes were sampled of which 50 samples from each market were collected and transported to the Microbiology laboratory of Bingham University for analysis. The samples were collected in sterile polythene bags using hand gloves.

2.2Sample Preparation and Culturing

The samples of spoiled tomatoes collected were crushed with a sterile mortar and pestle. The resulting tomato paste were first sieved using a sterile muslin cloth to remove lumps, and the supernatant was subjected to tenfold serial dilution (10^{-1}-10^{-10}) using sterile distilled water after which 1ml of the last diluent (10^{-10}) were inoculated using the pour plate technique on MaCconkey and Blood agar [11]. Inoculated plates were incubated for 24 hours at 37^0C after which pure colonies were sub-cultured by streak method.

2.3Identification of Bacteria Isolate.

Identification of bacteria isolates was done using gram staining technique and biochemical test (catalase, citrate, oxidase, urease and sugar fermentation test) [12].

Gram staining: fixation of smear was carried out by spreading loopful of isolate on a glass slide and passing it over low flame 3 times to heat fix. Smear was covered with 1 % crystal violet for 1 minute and washed off with tap water. Lugol's iodine solution was added and left for 1 minute and was washed off with tap water. 95 % ethanol was added for 10 seconds and washed off with tap water and counter stained with 2 % safranin for 30 seconds, washed off before being observed under light microscope. Gram positive isolate appeared purple while gram negative isolate appeared pink.

Catalase test: one drop of 3 % hydrogen peroxide (H_2O_2) was added to a loopful of each isolate. If the organism is catalase positive, it will produce hydrogen bubbles.

Citrate test: the isolates were inoculated on slants of Simmon's citrate agar then incubated at 37 °C for 24 hours. If the organism is citrate positive, the slant agar will change from green to blue.

Oxidase test: A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is oxidase positive, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Urease test: The surface of urea agar slant was streaked with portion of a well isolated colony, leaving the cap loosely tied and incubated at 35^0-37^0C in ambient air for 48hours to. It was examine for the development of pink color.

Sugar Fermentation: The ability of the bacteria isolates to ferment glucose, lactose, sucrose, and maltose were determined by growing each of the isolates in liquid standard medium containing 1.5g of peptone

water in 2g of each sugar. Durham-tubes were inverted into the culture tubes for gas collection. It was Incubated at 37 °C for 24 hours and uninoculated broths was use as control [13].

3.0 RESULTS AND DISCUSSION

3.1 Morphological and Gram Stain Identification of Bacteria Isolate

From the 50 samples of spoiled tomatoes collected from Masaka Market in Nasarawa State, Six (6) bacteria species were isolated, of which 4 of them grew on MaCconkey agar with a rod shape and were negative to Gram stain while 2 grew on Blood gar with a cocci shape and were positive to gram stain, as shown in table 1.
Base on 50 samples of spoilt tomatoes collected from Mararaba Market in Nasarawa State, 4 bacteria were isolated, out of which 3 grew on MaCconkey agar with a rod shape and were negative to gram stain while 1 grew on Blood agar with a cocci shape and was positive to gram stain, all of which are shown in table 1.

Table 1: Morphological and Gram Stain Identification of bacteria isolated from Spoilt tomatoes at Masaka and Maraba Market, Nasarawa State.

Location	Isolates	Colony Colour	SShape	Gram Stain
Masaka Market				
	1	Large colourless colonies with dark centers on MacConkey	Rods	Gram Negative
	2	Mucoid pinkish colony on MacConkey	Rods	Gram Negative
	3	Tiny slightly pinkish colonies on MacConkey	Rods	Gram Negative
	4	Pink mucoid colonies on MacConkey	Rods	Gram Negative
	5	Yellowish colonies on blood agar	Cocci	Gram Positive
	6	Yellowish-greenish colonies on blood agar	Cocci	Gram Positive
Mararaba Market				
	1	Large colourless colonies with dark centers on MacConkey	Rods	Gram negative
	2	Pink mucoid colonies on MacConkey	Rods	Gram negative
	3	Tiny slightly pinkish colonies on MacConkey	Rods	Gram Negative
	4	Yellowish colonies on blood agar	Cocci	Gram Positive

3.2 Biochemical Identification of Gram Negative Bacteria Isolates

Biochemical test based on standard bacteriological procedures were carried out on the 7 Gram negative bacteria isolated from Masaka and Mararaba Market, Nasarawa State. The first bacteria isolate from Masaka Market tested positive to citrate andcatalase tests, and negative to oxidase and urease tests. For

sugar fermentation test it was positive to glucose, maltose and sucrose while it tested negative to lactose, *Salmonella* specie was suspected. The second bacteria isolate tested positive to citrate, catalase and urease tests while it tested negative to oxidase test, and for sugar fermentation test it was positive to glucose, lactose, maltose and sucrose, *Klebsiella* specie was suspected.The third bacteria isolate tested negative to catalase and urease tests while it tested positive to oxidase and citrate test. For sugar fermentation test, it was positive to glucose, maltose, and sucrose while negative to lactose, *Vibrio* specie was suspected. The fourth bacteria isolate tested negative to citrate, oxidase and urease test, and for sugar fermentation test, it was positive to glucose and lactose, while it was negative to maltose and sucrose, *Escherichia coli* specie was suspected as shown in table 2.

For Mararaba Market, the first bacteria isolate tested positive to citrate and catalase tests, and negative to oxidase and urease tests. For sugar fermentation test it was positive to glucose, maltose and sucrose but tested negative to lactose, *Salmonella* specie was suspected. The second bacteria isolate tested negative to citrate,oxidase and urease test but tested positive to catalase test. For sugar fermentation test, it was positive to glucose and lactose, while tested negative to maltose and sucrose, *Escherichia coli* specie was suspected and the third bacteria isolate tested negative to catalase and urease tests but tested positive to oxidase and citrate test. For sugar fermentation test, it was positive to glucose, maltose, and sucrose and tested negative to lactose, *Vibrio* specie was suspected as also shown in table 2.

Table 2: Biochemical Identification of Gram Negative Bacteria Isolated from Spoilt Tomatoes at Masakaand Mararaba Market, Nasarawa State.

Location	Isolates	Citrate	Catalase	Oxidase	Urease	Sugar fermentation test				Probable bacteria
						Glucose	Lactose	Maltose	Sucrose	
Masaka Market										
	1	+	+	-	-	+	-	+	+	*Salmonella* spp.
	2	+	+	-	+	+	+	+	+	*Klebsiella* spp.
	3	+	-	+	-	+	-	+	+	*Vibrio* spp.
	4	-	+	-	-	+	+	-	-	*Escherichia coli* spp.
Mararaba Market										
	1	+	+	-	-	+	-	+	+	*Salmonella* spp.
	2	-	+	-	-	+	+	-	-	*Escherichia coli* spp.
	3	+	-	+	-	+	-	+	+	*Vibrio* spp.

KEY: (+) Positive Reaction; (−) Negative Reaction

Salmonella, Escherichia coli, Klebsiella and vibrio species were the gram negative bacteria isolated from both market which is almost similar to the work done by [7] and [14], and these gram negative bacteria are of public healthy significance because they are amongst the deadly food borne disease pathogens most especially *Salmonella* and *vibrio* species which can cause food borne related infections if few cells are ingested, although it is totally dependable on the age of the patient, and the health status of the host

(strength of the immune system). *Esherichia coli and Klebseilla* species can be very pathogenic as well because they are among the food borne disease pathogens implicated in the contamination of fresh produce like fruits and vegetables [15] and [16].
According to [17], the growth of the above mentioned organisms on tomatoes, most especially salmonella increases when the tomatoes are in contact with contaminated soil and environmentsuch as the dirty environment of both markets as captured in figure 1 and 2.
Fig.1 and Fig.2 are images of Masaka and Mararaba Market in Nasarawa State, showing the dirty environment where tomatoes are sold.


Fig.1. Masaka MarketFig. 2. Mararaba Market.

4.0 CONCLUSION

The gram negative bacteria associated with spoilage of tomatoes sold at Mararaba and Masaka Market New Karu Local Government of Nasarawa State are *Salmonella, Escherichia coli, Klebsiella and vibrio species,* thus the purchase and consumption of spoilt tomatoes at both markets could predisposes the consumer to frequent food borne infection by the above mentioned organisms.

Recommendation:It is therefore imperative that market environment which revolves around selling and storage of perishable foods be kept clean as a means to control contamination and proliferation of food-borne pathogens from infecting tomatoes and other perishable foods.

AUTHORS' CONTRIBUTIONS

Olokun Alexander Lanzema designed the study and performed the statistical analysis, Ajide Adeyoola Bukola wrote the protocol and the first draft of the manuscript while Ihuma Jerome Obo managed the literature and analyses of the study. Authors declared no competing interest

REFERENCES

1. Jay JM. Modern Food Microbiology. Aspen publishers, Inc. Gaithersburg, Maryland 2000. *A Wolters Kluwer Company*. www.asenpublishers.com Editorial Service: Joan Sesma. Library of

congress Catalog Card Number: 99-054735. ISBN: 0-8342-1671-X. Printed in the United States of America:2000

2. ACMSF (Advisory Committee on the Microbiological Safety of Food). *Microbial status of ready to eat fruit and vegetable*. Retrieve from http://www.food.gov.uk/multimedia.:2005
3. Andrew, F. The tomato in America: history, culture and cookery. Columbia, S.C, USA: *University of South Carolina Press*. 1994:ISBN 1-5700-3000-6
4. Njoku OF. Food Microbiology, McGrawHill Book Company:1991
5. Berdegue, J.A., F. Balsevich, L. Flores and T. Reardon, 2005. Central American Supermarket private standards of quality and safety in procurement of fresh fruits and vegetables, Food Policy, 30: 254-269
6. Beuchat, L.R., 2006. Vectors and conditions for pre-harvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. Britain food Journal, 108: 38-53
7. Oviness M., Chinkuli KM., Seter S., Mubbunu .L. Isolation, identification and characterisation of bacteria from ripe tomatoes grown under different agronomic conditions.*Asian Pac. J. Health Sci*; 2017:4(3):139-150.
8. De Rover C. (2001).Microbial safety evaluations and recommendations on fresh produce. Food Control 2001:9(6): 321-347
9. Ahmed RS, Abd El-Monem MA, Ali GA, Ahmed AH, Kareem MM. Prevalence of the Harmful Gram-Negative Bacteria in Ready-to-Eat Foods in Egypt, *Food and Public Health*, 2017:7(3):59-68.
10. Beuchat LR. Vectors and conditions for pre-harvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *Britain Food Journal*. 2006:108:38-53.
11. Ibrahim, M. and Sada, MD. Yeasts Associated with Spoilage of Some Selected Fruits in Sokoto Metropolis. Journal of advancement in medical and life sciences 2015:3:248-294
12. Cheesbrough M. District Laboratory Practice in Tropical Countries, University of Cambridge, Cambridge, UK, 2nd edition, 2005.
13. VashistHJ, Sharma DA, and Gupta AT. A Review on Commonly Used Biochemical Test For Bacteria. Innovare Journal of Life Science. 2013:1(1): 1-7
14. Ugwu OC, Chukwuezi FO and Ozougwu VE. Microbial Agents of Tomato Spoilage in Onitsha Metropolis. Advances in Biological Research 2014:8 (2): 87-93.
15. Adekanle MA, Effedua HI, Oritogun KS, Adesiji YO, And Ogunledun A. A Study Of Microbial Analysis Of Fresh Fruits And Vegetables In Sagamu Markets South-West, Nigeria. Agrosearch 2015:15(2): 1 - 12
16. Brigitte N and Van D. Evaluation of the microbial safety of commercially produced tomatoes in South Africa and the development of a novel enrichment broth for the identification of Escherichia coli O157:H7. University of Pretoria. 2015:1-184
17. Joshua BG, Nia AH, Amanda MS, and keith RS. Salmonella enteric contamination of market fresh tomatoes: a review. Journal of food protection, 2018: 81(7): 1193–1213