

Original Research Article

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

Comment [RJW1]: Put comma

Comment [RJW2]: Remove in

Comment [RJW3]: Put AREA after GOVERNMENT THEN PUT COMMA

Comment [RJW4]: Remove IN

ABSTRACT

Aims: The study is aimed at isolating and identifying the gram negative bacteria associated with spoilage of tomatoes at Mararaba and Masaka markets of Karu Local Government in Nasarawa State.

Study design: A cross-sectional study of gram negative bacteria associated with spoilage of tomatoes at Mararaba and Masaka markets of Karu Local Government in Nasarawa State.

Place and duration of study: 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 before inoculating on growth media 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*, *E. coli spp*, *Klebsiella spp* and *vibrio spp*, were the gram negative bacteria isolated from the samples collected from both market.

Conclusion: Tomatoes sold at poor hygienic environment are at higher risk of contamination by food-borne bacteria pathogens.

Comment [RJW5]: Put comma after the months

Comment [RJW6]: Serial dilution carried out using what? sterile Pepton water, sterile distilled water or what?????...culture inoculated on which type of media??????

Comment [RJW7]: Spp is not a name of microorganisms, why italicized???

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

1.0 INTRODUCTION

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

Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well-balanced diet which 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].

The vulnerability of the tomato to contamination by mostly *salmonella* and other major human pathogens that are common cause of foodborne illness has remained a mystery. If the illness is not detected early and controlled, it can cause death [5]. According to [6], vegetables and fruits have been associated with outbreaks of foodborne diseases in many countries. Interestingly, Gram-negative bacteria pathogens account for approximately 69% of the cases of bacterial food-borne disease [7].

Comment [RJW8]: Better written and italicised as *Salmonella*

Comment [RJW9]: separate

The contamination of tomato by micro-organism could be as a result of poor handling practices in the tomato production chain, storage condition, distribution, marketing practices and transportation [8]. **The aim of this study**.....

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Fresh Samples of spoiled tomatoes were collected from two major markets within Nasarawa metropolis; they include 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.

2.2 Sample Preparation and Culturing

The samples of spoiled tomatoes collected were crushed with a sterile mortar and pestle. The resulting tomato paste were first sieved to remove lumps and then subjected to tenfold serial dilution (10^{-1} - 10^{-10}) after which 1ml of the last diluent (10^{-10}) were inoculated using the pour plate technique on MacConkey and Blood agar [9]. They were incubated for 24 hours at 37°C after which pure colonies were sub-cultured by streak method.

2.3 Identification of Bacteria Isolate.

Identification of bacteria isolates was done using gram staining technique and biochemical test (catalase, citrate, oxidase and urease test) [10].

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. Smear was covered with 1 % crystal violet, Lugol's iodine solution and washed with 95 % ethanol and stained with 2 % safranin before being observed under light microscope.

Catalase test: one drop of 3 % hydrogen peroxide (H_2O_2) was added to a loopful of LAB culture.

And what happen??????

Citrate test: the LAB culture was inoculated on slants of Simmon's citrate agar then incubated at 37°C for 24 hours.

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° - 37°C in ambient air for 48hours to. It was examine for the development of pink color.

Comment [RJW10]: you short circuited the standard protocol....

Comment [RJW11]: give full mining at first mention

Comment [RJW12]: ?????????

Comment [RJW13]: Four biochemical test will NOT enable you identify the organisms. Is too shallow

3.0 RESULTS AND DISCUSSION

3.1 Morphological and Gram Stain Identification of Bacteria Isolate

Out of the 50 samples of spoiled tomatoes collected from Masaka Market of 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 agar with a cocci shape and were positive to Gram stain, as shown in table 1.

Table 1: Morphological and Gram Stain Identification of bacteria isolated from Spoilt tomatoes at Masaka market

Isolates	Colony color	shape	Gram stain
1	Large colorless 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

Also out of 50 samples of spoilt tomatoes collected from Mararaba Market of 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 as shown in table 2.

Table 2: Morphological and Gram Stain Identification of bacteria isolated from tomatoes at Mararaba market

Isolates	Colony color	shape	Gram stain
1	Large colorless 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 4 Gram negative bacteria isolated from Masaka Market of Nasarawa State of which the first bacteria isolate tested positive to citrate test and negative to catalase, oxidase and urease tests respectively, *Salmonella* *Salmonella* specie was suspected. The second bacteria isolate tested positive to citrate and urease test while it tested negative to catalase and oxidase test and *Klebsiella* specie was suspected. The third bacteria isolate tested negative to citrate, catalase and urease test while it tested positive to oxidase test, *Vibrio*

Comment [RJW14]: italicise

specie was suspected while the fourth bacteria isolate tested negative to all the test and *Escherichia coli* specie was suspected as shown in table 3.

Comment [RJW16]: italicise

Isolate	Citrate test	Catalase test	Oxidase test	Urease test	Probable bacteria
1	+	-	-	-	<i>Salmonella</i>
2	+	-	-	+	<i>Klebsiella</i>
3	-	-	+	-	<i>Vibrio spp</i>
4	-	-	-	-	<i>Escherichia coli</i>

Comment [RJW17]: donot italicise

Table 3: Biochemical Identification of Gram Negative Bacteria Isolated from Spoilt Tomatoes at Masaka Market

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

Biochemical test based on standard bacteriological procedures were also carried out on the 3 Gram negative bacteria isolated from Mararaba Market of Nasarawa State of which the first bacteria isolate tested positive to citrate test and negative to catalase, oxidase and urease test, *Salmonella* spp was suspected. The second bacteria isolate tested negative to all the test and *E. coli* spp was suspected while the third bacteria isolate tested negative to citrate, catalase and urease test while it tested positive to oxidase test and *vibrio* spp was suspected as shown in table 4.

Comment [RJW18]: italicise

Comment [RJW19]: i have not seen this COMBINATION before in Microbiology, how could you suspect only *E.coli* while the media you used was for lactose fermenters.

Comment [RJW20]: TREAT AS CORRECTED EARLIER

Table 4: Biochemical Identification of Gram Negative Bacteria Isolated from Spoilt Tomatoes at Mararaba Market

Isolate	Citrate test	Catalase test	Oxidase test	Urease test	Probable bacteria
1	+	-	-	-	<i>Salmonella</i>
2	-	-	-	-	<i>Escherichia coli</i>
3	-	-	+	-	<i>Vibrio spp</i>

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

Fig.1 and Fig.2 are images of Masaka and Mararaba Market of Nasarawa State, showing the dirty environment where tomatoes are sold.

Comment [RJW21]: I have not seen any discussion



Fig. 1. Masaka Market



Fig. 2. Mararaba Market.

4.0 CONCLUSION

The result obtained from this study, tomatoes sold at both market are contaminated with gram negative bacteria isolated during the course of this research and consumers are at risk of being exposed to with *Salmonella spp*, *E. coli spp*, *Klebsiella spp* and *vibrio spp.*, which are the leading cause of food-borne illness. 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.

Comment [RJW22]: Break into short sentences. Then bring out the achievements of this research

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. 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.
6. De Rover C. (2001).Microbial safety evaluations and recommendations on fresh produce. *Food Control* 2001;9(6): 321-347
7. 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.
8. 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.
9. Ibrahim, M. and Sada, MD (2015). Yeasts Associated with Spoilage of Some Selected Fruits in Sokoto Metropolis. *Journal of advancement in medical and life sciences* 2015;3:248-294
10. Cheesbrough M. *District Laboratory Practice in Tropical Countries*, University of Cambridge, Cambridge, UK, 2nd edition, 2005. View at Publisher · View at Google Scholar:2005

UNDER PEER REVIEW

