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Isolation and Identification of Bacteria Associated with Spoilt Tomato (*Lycopersicon esculentum*) Sold at Gombe Main Market, Gombe State

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ABSTRACT

Tomato (*Lycopersicon esculentum*) contains high water content making it prone microbial spoilage, thus causing a significant public health concern. This study is aimed at determining the several pathogenic bacteria linked with spoiled tomatoes sold in Gombe Main Market, Gombe State. A total of 50 spoilt tomatoes samples sold in Gombe Main Market were collected for analysis. Collected samples were cultured on Nutrient agar, Eosin Methylene Blue agar, Manitol Salt agar, *Samonella Shigella agar, and* Mueller Hinton agar. Five species of bacteria were isolated and identified based on their morphological and biochemical characteristics. The bacterial species isolated include: *Bacillus spp, Escherichia coli, Klebsiella aerogenes, Shigella* and *Staphyloccocus aureus*. The antibiotic sensitive pattern of bacterial isolates was determined using Kirby- Bauer disk-diffusion technique. Varying levels of antibiogram sensitivities and resistances were detected on all the bacteria isolated. The bacteria especially gram negative, were found to be key responsible for tomato spoilage. The occurrence of antibiotic resistant bacteria amid tomatoes is alarming on public health risks. Technological advancement of harvesting, packing, handling, storing and preservation might decrease tomato fruit losses and incessantly improve shelf life and quality.

Keywords: Antibiogram. pathogenic bacteria, public-health, and tomatoes,

INTRODUCTION

Tomato (*lycopersiconesculentum)*, is a palatable, berry plant in the *solanaceae* family often red colored berry fruit. It is a fragile perennial plant, cultivated as an annual crop, usually grown to about 1-5m in height (Wogu and Ofuase, 2014). The fruit is a structure that progresses from the ovary of the plant after fertilization, its flesh containing the pericarp walls. It is originated in South Africa in Peru/Ecuador region and was taken to the phillipines and Malaya in the 1650's. It is a true fruit consisting of seeds and moisture embedded in a gelatinous mass of lobular cavities which vary according to type. Its color ranges from green at tender age to yellow/orange or red at maturity depending on the predominant pigment that is Lycopene or B-carotene. Lately, there has been a surge in the need to isolate and identify the microorganisms related with the deterioration as a way of finding a means of mitigating it (Akinyele and Akinkumi, 2012).

Tomato is one of the most consumed fresh fruit globally as it offers a balanced diet that is rich in vitamins such as A,B, C and E; It contain carbohydrates such as fructose and glucose; Minerals (phosphorous, sodium, potassium, calcium, magnesium) as well as trace elements (iron, copper, Zinc) and Dietary fibers (Freeman and Reimers, 2015). Tomato fruit has 4.3% carbohydrate, 1% protein, 0.1%, 94% water, fat, 0.6% fibre and vitamins (Wogu and Ofuase, 2014). It is

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extensively used as condiment or as supplement in different part of the universe. It also improves the skin's capability to fight against dangerous ultraviolet rays. The consumption of tomato fruit is associated with decreased risk of breast cancer. Again, lycopene especially in cooked tomato help to prevent prostate cancer. Owing to its nutritious value, flavor, affordability, and availability, there has been an surge in demand by consumers (Behravesh *et al*., 2012).

Spoilage is any alteration in the condition of food in which the food becomes unfit for consumption (USDA, 2017). Vulnerability of tomato to bacteriological colonization is attributed to its chemical constituent like high sugar level, low pH (4.9-6.5) and its water activity ($p>0.99$) which support microbial growth in tomato thus, a basis of possible health hazard to human. This can cause severe health challenges like gastroenteritis, diarrhoea in man following ingestion (EUCAST, 2016). Tomatoes spoilage resulting from metabolic processes of bacteria, the sugar is promptly used up, converting it into acetic acid, lactic acid, alcohol, and carbon dioxide. The quantity of these substances hang on on the nature of bacteria that are most viable in the particular sample in question (Ogbomo, 2016). Typically, as spoilage progress, citric acid rapidly decomposed, so that its extent aids as a valuable index in ascertaining decomposition. It is easier to notice spoilage in tomato pulp or canned tomatoes, because when these products are wholesome they contain no volatile acids and the shape of the can is perfect. However, when spoiled volatile acids/gas are present (Wogu and Ofuase, 2014). The aim of this research is to isolate and identify bacteria associated with spoiled tomatoes sold in Gombe main market of Gombe State.

MATERIALS AND METHODS

Study Area

The samples used for this work were obtained from Gombe main market of Gombe State. Herwa Gana road Latitude: 10.298589, Latitude: 11.164799.

Sample Collection

A total of 50 spoilt tomatoes (5 each) were purchased at random from 10 different vendors in order to facilitate wider coverage of the study area. The samples were then aseptically transferred into sterile sample bags, labeled and transported to the Microbiology laboratory, reserved from dust and pest at room temperature.

Culture Media

The culture-media used for this research include: Nutrient Agar, Eosine Methylene Blue Agar, Manitol Salt Agar, *Salmonella Shigella* Agar, Mueller Hinton agar, and MIU medium.

Preparation of media

The various media were prepared based on the manufacturer's instructions. Media were prepared by weighing approximate amount of the powder into a conical flask then dissolved using distilled water. The flasks were then plugged with cotton wool surrounded with aluminium foil and sealed with masking tape and labeled appropriately. The media were then homogenized on hot plate before sterilizing in the autoclave at 121ºC for 15 minutes. The sterile media were allowed to cool to about 45ºC before being poured into sterile petri-dishes and allowed to set.

Isolation and Enumeration of Bacteria

Employing standard Microbiological technique (serial dilution), a tenfold dilution of 1ml of the tomato sample was carried out in 9ml of distilled water. Precisely, 1ml was

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pipette and mixed in another 9ml of sterile distilled water in a test-tube. The test-tube was vigorously shaken to homogenize. The exponential dilution continued up to the sixth factor. Then 1ml of the fifth factor was transferred aseptically and plated in triplicate. The poured plates were then allowed to set and incubated for 48 hours at 37º C. Discrete colonies that grow after incubation were counted and enumerated as colony forming unit (cfu/ml) after multiplying with the dilution factor (Chessbrough, 2006).

Subculture and Preservation of Isolates

Subculture

Pure isolates were collected by picking discrete colonies and subculturing them onto freshly prepared media in petri-dishes.

Preservation

Pure isolates were inoculated onto freshly prepared NA slants in flavor bottles for preservation. NA cultures were incubated for 24 hours in an incubator at 37ºC and then stored in the refrigerator at 4ºC.

Identification of Isolated Strains

The distinct colonies that grew in the pure culture plates were observed for;

I. Macroscopy

Morphology of the colonies such as shape, colour, texture, consistency, motility of the isolates were determined from the culture.

II. Microscopy

The colonies were Gram stained and subjected to different biochemical tests such as Motility test, Catalase test, Coagulase test, Urease test and, Indole test to identify the isolates.

Susceptibility Testing

Antibiotic sensitive of all the bacterial isolates were performed using Kirby-Bauer disk diffusion technique (CLSI, 2021). Amoxycillin(10μg), erythromycin(15μg), ceftriaxone(30μg), gentamicin(10μg), ciprofloxacin(5μg), and Chloramphenicol(30μg). These antibiotics are commonly used for the treatment of microbial infections in the general populace.

The result of the table below shows that sample 10 has the highest bacterial count of 11.3 (cfu/ml), followed by sample 9 (9.6 $x10^5$), sample 7 (9.0 $x10^5$), and sample 6 (8.6 $x10^5$). While sample 8 (1.6 $x10^5$) shows the least bacterial count and sample 1 too numerous to count.

Table 1: Shows the result of the total bacterial count for the organisms isolated from spoiled tomato

Sample Area Gombe Main Market	Average bacterial count (cfu/ml) NA
1st sample	TNTC
2nd sample	5.3 $x10^5$
3rd sample	6.3 $x10^5$
4th sample	3.3 $x10^5$
5th sample	3.0 $x10^5$
6th sample	8.6 $x10^5$
7th sample	9.0 $x10^5$
8th sample	1.6 $x10^5$
9th sample	9.6 $x10^5$
10th sample	11.3 $x10^5$

KEYTNTC: Too Numerous To Count. NA: Nutrient Agar

The result of the table below shows that *E. coli* has the highest frequency of (30%), followed by *Shigella* (23.3%), *Bacillus* (20%), *S.aureus* (16.7%) and the least is *Klebsiella* (10%).

Table 2: % Frequency of bacterial isolate

Isolate	Frequency	% frequency
E.coli	9	30
S.aureus	5	16.7
Bacillus	6	20
Shigella	7	23.3
Klebsiella	3	10
TOTAL	30	100

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Table 3: Cultural, morphological and biochemical characteristics of bacterial isolate from spoiled tomatoes

Cultural characteristic	Gram staining	Indole test	Urease test	Motility Test	Catalase	Coagulase	Isolate
Greenish metallic sheen	- r od	+	-	+	+	-	*E.coli*
Yellowish colony	+ cocci	-	+	-	+	+	*S.aureus*
Gray white, granular colony	+ rod	-	-	+	+	-	*Bacillus*
Colourless colonies	- rod	-	-	-	+	+	*Shigella*
Large mucoid colonies	- rod	-	+	-	+	-	*Klebsiella*

KEY: +: Positive, - : Negative

The result of the table below shows that *S.aureus* is the most sensitive to antibiotics used in this research while *Shigella* and *E. coli* represent the higest level of resistance and intermediate.

Table 4: Patterns of antibiotic sensitivity of bacterial isolates in spoilt tomato fruit samples from Gombe Main Market, Gombe State.

	SENSITIVE (mm)					**RESISTANT (mm)**				
BACTERIA	**CIP**	**CHL**	**GEN**	**ERY**	**CTR**	**GEN**	**ERY**	**CTR**	**AMX**	**AUG**
S. aureus	30	22	19	23				2		
E. coli	28	26			18	11	2		2	
Klebsiella	24		19		22		0		2	
Shigella			19				0	9	0	3
Bacillus	29	22						0	10	0

KEY: Antibiotics: CIP = Ciprofloxacin, CHL =Chloramphenicol, GEN = Gentamycin, ERY = Erythromycin, CTR = Ceftriaxone, AMX = Amoxycilin, AUG = Augmentin.
Test Results: S= Sensitive, R= Resistance.

DISCUSSION

Tomatoes harbor an broad variety of bacterial contaminants (Long *et al*., 2012). The main bacterial species that are largely present on tomatoes comprise species of *Pseudomonas spp., Bacillus spp., Enterobacter spp., Staphylococcus spp., Streptococcus spp., Lactobacillus spp., Leuconostoc spp*. Several tomatoes are not often fresh as they remain in the market for lengthy periods causing their spoilage. Furthermore, most of the vegetables are often cultivate by irrigation, most water used in watering these plants are contaminated containing enteric bacteria, viruses, protozoa, or helminthes, thus increasing the possibility of isolating pathogens from harvested tomatoes. Typically, fresh fruits are surrounded by natural protective barrier (skin) that offer protection against most plant spoilage and pathogenic microorganisms. Yet, this protection may be disrupted by mechanical injury during harvest, post-harvest, handling and distribution exposing the fruits to microbial contamination. (Wogu and Ofuase, 2014).

The microbes present in the spoilt tomato fruits were identified based on their cultural, morphology and biochemical characteristics. The characterization and identification of the bacterial isolates are displayed in Table 3. The bacteria isolated were: *Esherichia coli, Bacillus spp., klebsiella spp., Staphylococcus aureus, and shigella spp*. based on their morphological and biochemical characteristics. The occurrence of *Escherichia coli* and *Staphylococcus aureus* in this study

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proven the findings reported by Bello *et al*. (2016). Isolation of the soil bacteria *Bacillus spp*. from the tomato samples is an indication of opportunistic contamination. Similarly, the existence of *Staphylococcus aureus* which are considered to be associated with fecal matter, and *E. coli* which is a normal human flora revealed that the tomato samples were contaminated via poor human hygiene and handling processes (Wogu and Ofuase, 2014).

In this study, *E. coli* (30%) was the most prevalent bacteria isolated. *Klebsiella* being the least (10%), as the study of Ugwu *et al*. (2014) in Nigeria which also documented 8.9% in spoilt tomatoes. Similarly, Wogu and Ofuase (2014) reported 1.6% for *Klebsiella* sp. The unpredictable occurrence of *klebsiella* isolates in the different areas could be attributed different human activities related with postharvest practices on the tomatoes before displaying the fruit for sale. In addition, *Klebsiella*spp., are omnipresent organisms that is found in all environment, including in animals, and humans (Pigott, 2018). This bacterium might have contaminated the tomatoes via mechanical injury during postharvest or poor handing, transportation and storage facilities Lemma *et al*. (2014). *Bacillus* sp. isolated in this study accounted for 20%, which is lower than 59.1%r stated by Wogu and Ofuase (2014). The difference in occurrence may be related with varying frequency of *Bacillus* sp. spores in the environment (Obieze *et al*., 2011). In addition, *Bacillus* sp. are resilient due to endospore which make resistant to high temperatures of the sun's ultraviolet rays, hence their bacterial load in the tomatoes.

The presence of *Shigella* sp. (23.3%) in the contaminated tomatoes samples suggesting that the tomatoes may have been exposed to faecal-contaminated water or manure during cultivation (Chuku *et al*., 2014). *Shigella* spp. isolated in this study is in contrast to Adebayo-Tayo *et al*. (2012) which reported higher *Shigella* sp. (30%) The contamination of tomatoes by these organisms may be attributed to poor or improper hygiene measure prior to handling of the tomatoes (Rahman and Noor, 2012).

Furthermore, most of the isolates were sensitive to Chloramphenicol, but none of the isolates expressed resistance to ciprofloxacin. However, resistance was observed for amoxycillin, augmentine, and erythromycin. The varying antibiotic prevalence has been previously reported by (Wogu and Ofuase, 2014) in a previous study on tomatoes in Benin City, Nigeria. The presence of bacteria with antibiotic resistance associated with tomatoes sampled in this study highlights the potential risk of tomatoes to consumers.

CONCLUSION

The numerous bacterial species isolated and identified in this present study reveal that bacterial spoilage on tomatoes can be a pose risk and health challenge to consumers. Spoilage or contamination by bacteria can cause illness like food poisoning and food-borne illnesses. Furthermore, the result of this study also indicate that microorganisms responsible for spoilage of fruits can penetrate into fruit during the processes of cultivating, harvesting, grading and packaging due to injury resulting mechanical damage. Owing to the severe nature of infection caused by these pathogenic isolates, it is pertinent to enlighten the public to discourage them from purchasing spoilt tomatoes as they can predispose the general public to infection.

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