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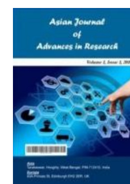

BACTERIA SCREENING OF TOMATOES (*Solanum lycopersicum*) SOLD IN SELECTED MARKETS IN ANAMBRA STATE, NIGERIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author ECF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOJ managed the analyses of the study. Author NNE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The high nutritional values of tomatoes (*Solanum lycopersicum)* have made them an important vegetable crop. Tomatoes are susceptible to various infections ranging from viruses to bacterial and fungal diseases, like other crops. Its susceptibility toward fungal attacks is due to its property to bear a succulent fruit. This study aims to identify the bacteria that cause the seed tomatoes to spoil in various markets in Anambra state (Eke Awka, Ifite Awka, Afia Nkpor, Afor Nkpor, and Onitsha main market). They were then transported to a Alpah research facility in Awka, for analysis. The bacteria that cause the seed tomatoes to spoil in these markets were identified using the standard procedure. These included *Salmonella*, *Klebsiella*, and *Escherichia coli*. These bacteria isolated were further subjected to antibiotic agents to know at which level they are likely to be more susceptible or resistant or intermediate to antibiotic agents. The Antibacterial susceptibility profile of selected antibiotics against the bacteria isolates indicated that these antibiotics were effective against the bacterial isolates from the tomatoes samples gotten from different markets at different concentrations. The presence and activities of these microbial growths and contaminations on tomatoes cause spoilage, reduce shelf life, and thus lead to loss and wastage of products which have a remarkable economic impact.

Keywords: Tomato; bacteria; antibiotics.

INTRODUCTION

As a nutritious food crop, tomatoes (*Solanum lycopersicum*) are known for their high nutritional value. However, they are also susceptible to various diseases, which can limit their production. One of these is the fungi that can attack its fruit [1].

Plant pathogens have been identified as the main causes of various diseases in tomatoes. These include

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the *Botrytis cinerea*, *Fusarium solani*, *Alternaria solani*, *Verticillium sp* and the *Rhizoctonia solani*. Meanwhile, some of the most severe and destructive diseases affecting both field and greenhouse-grown crops are the bacterial diseases of tomatoes. They have the capability to cause localized epidemics under moist field conditions affecting young developing fruit; in the greenhouse can cause total crop losses [2].

Bacterial infection on tomatoes constitutes a major problem for farmers in many countries. This problem includes poor yield and loss of quantity and quality of the fruits. Several studies on bacterial screening of tomatoes have been reported in Nigeria and other parts of the world. There is a dearth of information on the status of bacterial infection of tomatoes in Awka, Anambra State, Nigeria. To identify the risk, prevent and control diseases, a comprehensive understanding of the type of phytopathogen of tomato in a particular area is crucial. Conventional methods of preventing fungal infections include the use of chemical fungicides. However, due to the emergence of resistance races and the increasing environmental pollution, seed bio priming is becoming an alternative [3].

This study aims to determine the possible bacterial infection of tomato seed in Anambra State, Nigeria. To identify (check or screen) bacterial infection of tomato seed.

2. MATERIALS AND METHODS

2.1 Sample Collection

The tomato samples were purchased from five (5) markets in Anambra state (Eke Awka, Ifite Awka, Afia Nkpor, Afor Nkpor, and Onitsha main market). The samples will be collected into a sterile container and labeled respectively. Laboratory and other facilities used in the practical work were obtained from Alpha research laboratory Awka, Anambra state.

2.2 Sample Preparation

The fresh tomatoes were washed with sterile water before the tomatoes were cut into two halves and the seeds were carefully removed, washed with normal saline, and dried at room temperature.

2.3 Analysis of Sample

2.3.1 Isolation of bacteria

The method was used to isolate the bacteria from the tomato seeds using nutrient agar and EMB agar. The (One gram) seeds were aseptically collected and then serially diluted in normal saline In order to determine the total number of aerobic heterotrophic bacteria and fecal coliforms in the sample, the agar was inoculated with a combination of nutrients agar and EMB, they were incubated at 350ºC for 24 hours.

2.3.2 Total plate count of bacteria (CFU/ml)

The microbial load in agar plate samples was calculated using a formula.

Cfu/ml = {(No. of colonies X dilution factor) / volume of inoculums]

2.3.3 Purification of isolates

The selected colonies were then subcultured on nutrient agar plates for 24 hours. They were then subjected to biochemical analysis and microscopic characterization.

2.3.4 Identification of microorganisms

a. **Morphological identification:** The isolated bacteria were identified based on motility and Gram's-staining.
b. **Gram's staining:** The samples were stained according to Gram's techniques. A thin smear was prepared on a glass slide and air dried, and then heated to a high temperature. The smear was then covered with iodine for 60 seconds. It was then decolored using 70% ethanol and then washed under tap water. It was then counterstained with safarin for 30 seconds. The slide was then dried using a filter paper. The cells were then examined using the light microscope's oil immersion objective lens. The colors of the organisms were also studied. The purple color of the bacteria is due to their growth rate, while the pink color is due to their shape.

Motility test

The stabbing technique was used to carry out this test. Test tubes containing sterilized Sim Agar were prepared. A sterilized inoculating needle was used to pick up isolates from their pure cultures. Each test tube was stabbed with the needle rubbed with each isolate in the middle. The test tubes were then incubated at 370ºC for 24hours. After 24 hours, the tubes were observed for the motility of the isolates. A motile isolate usually grows away from the point where the medium was stabbed.

Biochemical Identification: The isolated bacterial colonies were confirmed by Biochemical kits (Universal Food pathogen Identification Disc, Hi2TMEnterobacteriaciae Identification Kit, and TSI test) and the results were interpreted as per the interpretation chart and identification index following kit protocol [4].

2.3.5 Biochemical tests

Urease Test

The purpose of this test was to demonstrate the ability of the organisms to produce Urease, which is an enzyme that breaks down urea. A change in the color of the urea-agar after 37°C was confirmed by the test.

Catalase Test

The objective of the test was to determine which of the organisms could produce oxygen from hydrogen peroxide by catalase.

A colony was then placed into a glass slide. After a drop of 3% hydrogen peroxide, the colony produced gas bubbles. The reaction was then confirmed by the presence of catalase.

Methyl Red Test

The purpose of this test was to determine which of the various strains could produce and maintain a stable acid product after glucose fermentation. It is usually used to identify the *enterobacteriaceae* and to differentiate them from other bacteria. The culture was placed in a warm environment at 37°C for 48 hours. After 5 drops of the methyl red reagent, the red color of the culture immediately changed.

Voges -Proskeur Test (V.P. test)

This test was used to identify the organisms that can produce acetyl methyl carbinol, which is an essential component of carbohydrate metabolism. It is commonly used to differentiate between gram-negative and non-germ-negative organisms. Inoculated glucose broth was prepared with the test organism at a temperature of 370ºC for 3 days. A combination of naphthol and sodium hydroxide solution was added to the mixture and allowed to stand for 1 hour.

Indole Test

The purpose of this test was to determine which of the various strains can split indole from tryptophan in the presence of buffered peptone water. It is commonly used to differentiate Gram-negative and Bacilli. The culture tubes were incubated at 37°C for 48 hours. After 4 drops of Kovac reagent, the positive test was detected by a red color around the upper part of the test tube.

Citrate Utilization Test

The purpose of this test was to identify the various types of bacteria that can utilize citrate as their sole source of carbon for their metabolism. The medium used for this was Simon's citrate. The agar was inoculated with the young cultures of the various species. Inoculated tubes were placed in a Petri dish and subjected to a temperature of 37°C for about 24 hours. The resulting change in color indicated a positive result.

Coagulase Test

The coagulase test is used to identify the presence of a type of *Staphylococcus aureus*. It can be performed using the presence of a certain protein called coagulase. The method of Barry et al., [5] was employed.

2.4 Procedure

1. A very homogeneous suspension of the inoculated inoculum was mixed with a drop of normal saline.
2. A loopful of rabbit plasma was then added to the suspension and thoroughly mixed for 5 seconds.
3. A control was set up in the same manner without blood plasma.
4. Coagulase positive staphylococci showed clumping or agglutination within 5-15 seconds while negative suspension showed no clumping.

Oxidase Test

This was carried out to identify bacterial species that will produce the cytochrome oxidase enzyme.

A piece of filter paper was placed in a clean Petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test organism were collected using a glass rod and smeared on the filter paper and observed. Blue-purple color within few a seconds showed a positive test.

2.5 Antimicrobial Screening Tests

Antibiogram of the selected isolates from tomato seed samples in this study was ascertained on Mueller-Hinton agar using the Kirby Bauer disc diffusion method [6]. A total of 12 antibiotics corresponding to

drugs most commonly used in the treatment of human and animal infections caused by gram-negative and gram-positive bacteria were employed in this study. The antibiotics and their concentration included Augumentin (βlactams) 30 µg, Streptomycin (Aminoglycosides) 10 µg, Ciprofloxacin (Flouroquinolones) 10 µg, Gentamycin (Aminoglycosides) 10 µg, Rifampicin (Ansamycin) 20 µg, Ofloxacin (Quinolones) 10 µg, Norfloxacin (flouroquinolones) 10 µg, Erythromycin (Macrolides) 30 µg, Pefloxacin (Quinolones) 10 µg, lincocin (lincosamide) 20µg, Chloramphenicol (phenicols) 30 µg, Cotrimoxazole (sulphonamides) 30µg. Inoculated overnight cultures of the bacteria were inoculated into a solution of peptone water and incubated at room temperature for several hours. The agar plates were then uniformly inoculated by spotting the culture of the bacteria on the plate. For the study, the plates were placed on agar medium and subjected to various conditions. The plates were then incubated at a temperature of 370ºC for 24 hours. A zone of growth was measured around each disc. The degree of susceptibility of the bacteria to the various antibiotics was determined by the British Society for Antimicrobial Chemotherapy and a laboratory standard institute.

2.6 Data Analysis

The data was analyzed using a statistical program known as ANOVA. The results indicated that the P values less than 0.05 were statistically significant.

3. RESULTS

Five bacteria were identified from the tomato samples. These include *Klebsiella*, *Staphylococcus sp*, *Escherichia coli*, and *Pseudomonas*. These bacteria were identified using their morphology (Table 1).

Table 1. Morphological and biochemical characteristics of isolates

Parameters	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Colony Characterization	Milkish irregular shape with flat elevation	Yellowish circular with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation	Yellowish irregular shape with flat elevation
Cell characterization	Coci in clusters	Short rods in singles	Rods in clusters	Cocci in clusters	Cocci in clusters
Gram's Test	+	_	_	+	+
Motility Test	_	_	+	+	+
Catalase	_	+	+	+	+
Coagulase	_	_	_	+	+
Citrate	_	_	_	+	+
Indole	_	+	_	+	+
Oxidase	_	_	+	_	_
Urease	+	+	+	+	+
Probable organism	***Klebsiella spp***	***Bacillus spp***	***E- coli***	***Staphylococcus spp***	***Pseudomonas spp***

Table 2. Total bacterial count and faecal coliform bacterial (FCB) counts of tomato seeds

Sample site	Total Bacterial count (cfu/ml)	Coliform bacteria counts (cfu/ml)
Eke Awka	$90 \times 10^4 \pm 0.127^b$	$59 \times 10^4 \pm 0.127^a$
Ifite Awka	$60 \times 10^4 \pm 0.127^d$	$55 \times 10^4 \pm 0.127^b$
Afia Nkpor	$50 \times 10^4 \pm 0.127^e$	$53 \times 10^4 \pm 0.127^c$
Afor Nkpor	$70 \times 10^4 \pm 0.127^c$	$51 \times 10^4 \pm 0.127^c$
Onitsha market	$135 \times 10^4 \pm 0.127^a$	$37 \times 10^4 \pm 0.127^d$

**Values are mean scores ± Standard deviation of three (3) replicates*
**Data in the same column bearing different superscripts differ significantly ($p < 0.05$)*

Table 3. Antibiotic susceptibility pattern of the bacterial isolate

		Antibiotics sensitivity profile											
Site	**Isolates**	**CN**	**S**	**LC**	**CPX**	**RX**	**E**	**NOR**	**CH**	**OFX**	**PEF**	**AU**	**SXT**
Eke Awka 1	*Klebsiella* sp.	S	S	R	S	R	I	R	R	R	R	R	I
	bacillus	S	S	I	S	I	R	S	S	S	R	R	R
	Staphylococcus sp.	R	R	R	R	R	R	R	R	S	R	R	R
Ifite awka	*Staphylococcus* sp.	R	R	S	I	R	R	R	R	I	S	R	R
	Escherichia coli	S	R	S	S	R	R	S	I	S	S	R	S
	Klebsiella sp.	S	R	R	R	R	R	R	R	S	S	R	R
	Bacillus sp.	I	R	R	S	R	R	S	R	S	I	R	R
Afia Nkpor													
	Bacillus sp	R	S	R	R	R	R	R	R	S	R	R	R
	Klebsiella sp.	S	S	I	S	R	S	S	S	S	S	S	S
Afor Nkpor													
	Bacillus sp.	R	S	R	R	R	R	R	R	S	R	R	R
	Klebsiella sp.	S	S	I	S	R	S	S	S	S	S	S	S
Onitsha main market 3													
	Escherichia coli	R	R	R	S	R	R	R	S	S	R	R	R
	Salmonella sp.	R	S	R	S	R	R	R	R	I	R	R	R
	Bacillus sp.	S	R	I	S	R	S	R	R	S	R	R	R

***N/B: R** = Resistant, **I** = Intermediate, **S** = Susceptible;*

*Antibiotics used: **CN** - Gentamycin; **S** – Streptomycin; **LC** –Lincocin; **CPX**-Ciprofloxacin; **RX** – Rifampicin; **E** – Erythromycin; **NOR** - Norfloxacin; **CH**-Chloramphenicol; **OFX**-Ofloxacin; **PEF** - Pefloxacin; **AU**-Augmentin; **SXT**-Cotrimoxazole*

Table 2 above showed the mean total bacterial count (TBC) and Faecal coliform bacterial (FCB) in each tomatoes sample. Onitsha market had the highest bacterial counts (135 x 10^4 $\pm$ 0.127^a) while Afia Nkpor (50 x 10^4 $\pm$ 0.127^e) had the least bacterial counts on TBC. Eke Awka (59 x 10^4 $\pm$ 0.127^a) had the highest Coliform bacterial counts while Onitsha market (37 x 10^4 $\pm$ 0.127^d) had the least Coliform bacterial counts. The table above showed that there were significant differences in bacteria count (mean ISD).

From the above table, the results of antimicrobial screening showed that various bacteria isolates are likely the most susceptible or intermediate and resistant to the antibiotics. The Antibacterial susceptibility profile of selected antibiotics against the bacteria isolates showed that *Salmonella sp* was susceptible to Streptomycin, and Ciprofloxacin, at different concentrations in the various samples. *Escherichia coli* was susceptible to Gentamycin, Lincocin, Ciprofloxacin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Pefloxacin, and Cotrimaxazole. *Klebsiella sp.* was susceptible to Ciprofloxacin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Augmentin, cotrimaxazole, Erythromycin and Norfloxacin. *Bacillus* species were susceptible to Ciprofloxacin, Chloramphenicol, Ofloxacin, and Norfloxacin. *Staphylococcus aureus* was susceptible to Ofloxacin and Pefloxacin, therefore making these antibiotics effective against the bacterial isolates from five tomatoes samples at different concentrations.

4. DISCUSSION AND CONCLUSION

4.1 Discussion

Staphylococcus aureus, *Klebsiella sp, Salmonella sp*, *Bacillus sp*, and *Escherichia coli were* the bacteria isolated from the tomatoes samples from the different locations. These isolates were similar to that study conducted by Ogundipe et al., [7], Wogu and Ofuase [8] and Ghosh [9] who associated bacteria as organisms causing spoilage in tomatoes. Fecal contamination due to poor hygienic practices by the farmers and /or the sellers resulted in the occurrence of bacteria species in these samples [10]. Antibacterial susceptibility profile of selected antibiotics against the bacteria isolates indicated *Salmonella sp* was susceptible to Streptomycin, and Ciprofloxacin, at different concentrations in the various samples. *Escherichia coli* was susceptible to Gentamycin, Lincocin, Ciprofloxacin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Pefloxacin, and Cotrimaxazole. *Klebsiella sp.* was susceptible to Ciprofloxacin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Augmentin, cotrimoxazole, Erythromycin, and Norfloxacin. *Bacillus* species were susceptible to Ciprofloxacin, Chloramphenicol, Ofloxacin, and Norfloxacin, and *Staphylococcus aureus* was

susceptible to Ofloxacin and Pefloxacin, thus making these antibiotics effective against the bacterial isolates from the tomatoes samples gotten from the five different markets at different concentrations. This was also reported by Chuku et al. [11].

The microbial proliferation and contamination of tomatoes cause spoilage, decreased shelf life, and also decreased sensory appeal, leading to loss and wastage of products which have a significant financial effect [12].

4.2 Conclusion

The bacterial screening of tomatoes seed for analysis indicates that bacteria are one of the very few microorganisms that cause the spoilage of tomatoes. The bacteria associated with tomato spoilage were *Staphylococcus aureus*, *Klebsiella sp, Salmonella sp*, *Bacillus sp*, and *Escherichia coli*. The Antibiogram of selected antibiotics, against the bacteria, isolates indicated some were susceptible, intermediate, and resistant to antibiotics agents used.

4.3 Recommendation

It is recommended that;

1. The thorough washing of harvested tomatoes with clean or chlorinated water, proper cleaning, and sanitation of warehouses, and disinfection of packaging containers, proper handling of the vegetable during harvest should be done to prevent bruises and scars or other mechanical injuries.
2. The inhibition of bacteria by lowering storage temperature through storage under refrigeration of less than 100C but not freezing and the use of appropriate antimicrobial agents when stored by drying is encouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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