

Research Article

# Assessment of Bacterial Contamination in Ready-to-Eat Fruits and Vegetables Sold at Oja-Oba Market, Ilorin, Nigeria

# \*Ajiboye, A.E. and Emmanuel T.O.

Department of Bioscience and Biotechnology, Microbiology Unit, Kwara State University, Malete, Nigeria

#### ABSTRACT

Assessment of bacterial contamination was carried out on ready to eat fruits and vegetables sold in Oja-Oba market, Ilorin. Nine (9) samples of two (2) different fruits (watermelon & pawpaw) and vegetable (carrot) were collected randomly from different stationery vendors. Analyses of total bacterial count was carried done on all samples, selective/differential media was used to enumerate total coliform count, total *Staphylococcus* count and total *Salmonella* count using 10-fold serial dilution and plate count method. Pure colonies were isolated using streak plate method and subjected to biochemical test using standard procedure. Ten (10) antibiotics were used for susceptibility test against the biochemical tests using disk diffusion method. Mean microbial load ranged from  $60.17 \pm 3.10 \times 10^4 - 158.67 \pm 6.90 \times 10^4$  cfu/ml for vendor A;  $61.83 \pm 2.60 \times 10^4 - 144.33 \pm 4.24 \times 10^4$  cfu/ml for vendor B and  $56.83 \pm 3.53 \times 10^4 - 88.50 \pm 3.10 \times 10^4$  cfu/ml for vendor C. Total coliform count ranged from  $7.80 \pm 1.10 \times 10^4 - 26.70 \pm 2.82 \times 10^4$  cfu/ml, total *Staphylococcus* count ranged from  $5.00 \pm 0.24 \times 10^4 - 21.17 \pm 3.06 \times 10^4$  cfu/ml and total *Salmonella* count ranged from  $8.84 \pm 1.18 \times 10^4 - 11.67 \pm 1.41 \times 10^4$  cfu/ml. Antibiotics susceptibility test ranged from  $10.00 \pm 0.00 - 26.50 \pm 0.21$ mm. Ciprofloxacin and gentamycin had an average diameter of zone of inhibition at  $24.50 \pm 0.71$  and  $26.50 \pm 0.21$ mm respectively. The analysis has shown that ready to eat fruits and vegetables sold in Oja-Oba Market contain considerable numbers of pathogenic bacteria.

Keywords: Oja-Oba Market, Fruits, Vegetables, Bacterial isolates, Antibiotics

\*Author for correspondence: Email: adeyinka.ajiboye@kwasu.edu.ng; Tel: +234-08061102397

Received: March, 2020; Accepted: December, 2020

# INTRODUCTION

The consumption of fruits and vegetables have notable beneficial effect as it contains essential nutrients that support good health (Liu 2013), which include fiber, vitamins and essential minerals amongst others. Consumers of fresh fruits and vegetables are usually in a state of good health, hence the several health benefits being enjoyed (Adebolu and Ifesan, 2019).

There is a drastic reduction of incidences of acute and chronic diseases as a result of the intake of fruits and vegetables (Boeing *et al.*, 2012). There is considerable information on the need to consume on a daily basis fruits and vegetables as essential part of our diet, and it is also known that there could be development of poor health and increased incidences of disease due to insufficient consumption (Aune *et al.*, 2017). However, the intake of raw vegetables without proper washing is a great concern because it has been proved to harbor microbes (WHO, 2015). It has also been documented by Temgoua *et al.*, (2015) and Adams and Moss (2018) that contamination could arise from poor or lack of basic standard

systems deployed during post harvest transportation, handling and packaging.

Fruits and vegetables could be the sources of contamination of food preparation areas (Altieri and Nicholls, 2017). Ready to-eat fruits and vegetables may be sliced, peeled, shredded and washed/unwashed (Francis et al., 2012). The destruction of surface cells during processing can cause an exposure of the produce for the entry of microorganism which utilizes the readily available nutrients compared to intact produce. In addition, high water activity of many fruits and approximately neutral pH of vegetables encourages the rapid growth of microbes (Qadri et al., 2015). Members of the Enterobacteriaceae is often associated with contamination of fruits and vegetables due to activities and processes involved from cultivation and post harvest operations. Varieties of fruits and vegetables have been implicated as a source of Salmonella infection; most commonly are tomatoes, lettuce and watermelon. Salmonella sp. Has been found on fresh produce such as lettuce, cauliflower, spinach, mushrooms and mustard cress (Mritunjay and Kumar, 2015).

Generally, most of the street vendors that hawk ready to eat fruits and vegetables are not monitored by any food protection agency which means the risk of consuming fresh produce contaminated with infectious agent such as *Escherichia*, *Salmonella* and other parasite is high (Orji *et al.*, 2017). The ability of some certain bacteria to produce toxin causing food poisoning has also implicated Staphylococcus as one of the prevalent agent responsible for infection and can be transmitted from person to person (foster and McDevitt, 2013). The entirely study was aimed at assessing bacterial contamination in ready to eat fruits and vegetables sold at Oja-Oba Market, Ilorin.

# MATERIALS AND METHODS

**Collection of samples:** Fruits of two different types {Water melon (*Citrullus lanatus*), pawpaw (*Carica papaya*)} and Vegetable {Carrot (*Daucus carota*)} were collected from 3 different stationery vendors in Oja-Oba market. The two fruits were sliced ready to eat fruits. The total of all samples were three (3) and these was collected in sterile polytene bag and ice cooler at about 10:00-11:00 hours. It was transported immediately for processing in the laboratory.

**Preparation of Culture Media**: All media (Nutrient agar, MaConkey agar, Eosin methlenyne blue agar, Manitol salt agar, and Mueller Hinton agar used for culturing were prepared according to standard specification by the manufacturer and were sterilized at 121 °C for 15 minutes. Salmonella Shigella agar, which does not require autoclaving, was sterilized by boiling for 15 minutes.

**Preparation of Sample:** Ten grams (10g) of each sample was measured with the aid of a weighing balance, it was pressed using a sterile pestle and transferred into a sterile conical flask. A measuring cylinder was used to obtain 90 ml of sterile distilled water and this was introduced into the conical flask containing different sample.

**Preparation of Serial dilution:** Serial dilution of the original stock culture was done in five (5) test tubes. Nine (9) ml of distilled water was introduced into each test tubes using a measuring cylinder and transferred into all test tube. The test-tubes were corked with cotton wool and placed in an autoclaving for sterilization at 121 °C for 15 minutes and 15 Ibs. The distilled water was allowed to cool and 1 ml of the original stock sample was introduced into the first 9 ml sterile distilled water in test-tube to give a 10-fold serial dilution. This was also labelled 1/10 and the process was repeated on all test-tubes until dilution factor of 1/100000 was obtained (Kaur and Rai, 2015).

**Isolation and Enumeration of Bacteria:** About  $0.1 \text{ ml of } 10^{-3}$  dilution for each sample was inoculated on the different solidified and sterilized agar plates. The inoculums on each plate was spread using a sterile glass rod and the plates were inverted and placed in an incubator for 24-48 hours at 37°C. Enumeration of total bacteria count was done using the plate count method, colonies present were counted and recorded to get the total colony count in cfu/ml while sub – culturing of each isolates was done until pure colonies was obtained. Pure

cultures were then refrigerated at 4 °C. The method of Karoki *et al.* (2018) was used to characterize isolates using their macroscopic, elevation of colony and biochemical characteristics.

**Identification of Bacterial Isolate:** Identification of bacterial isolates was done using Gram stain technique and biochemical test such as catalase, indole, citrate utilization, oxidase, methyl red and voges- proskauer test.

**Catalase Test:** Three (3) % H<sub>2</sub>O<sub>2</sub> was introduced unto a clean grease free slide. A smear of loop full bacteria was made. Formation of bubbles was observed for each bacterial isolate (Mahon, 2011).

**Indole Test:** Peptone broth was prepared by weighing (15) ml of peptone broth in a test tube and sterilized in an autoclave at  $121^{\circ}$ C for 15 minutes at 15 ibs pressure. A loop full of bacteria culture was inoculated in broth and incubated for 24-48 hours at 37 °C. Two (2) drops of Kovac's reagent was dispensed into the test tubes and mixed together after sterilization (Abiola and Oyetayo, 2016).

**Oxidase Test:** Two (2) drops of oxidase reagent was placed onto whatman filter paper and a smear of bacteria culture was made from a 24 hours old nutrient agar plate. The formation of Bubbles or effervescence was observed for positive result ((Shields *et al.*, 2013).

**Citrate Utilization Test:** Two (2) grams of Sodiun Citrate, 5 g Sodium Chloride, 1 g Dipotassium Phosphate, 1 g Ammonium Dihydrogen Phosphate, 0.08 g Bromothylmol Blue, 0.2 g Magnesium Sulphate and 15g agar was mixed together and 1000ml sterile distilled water was dispensed in the same mixture. The pH was adjusted to 6.9 and gentle heat was applied to dissolve agar. About 3-4 ml was collected in glass bottles and sterilized at 121 °C for 15 minutes in an autoclave. This was cooled in a slant bottles and inoculums was smeared onto the surface of the slant (Chester and Copper, 2019).

**Methyl Red Test:** The bacteria culture was inoculated into a fresh sterile broth medium and incubated at 37 °C for 48 hours. A sterile pipette was used to dispense 5 drops of Methyl red reagent into the broth culture and colour change was observed (McDevitt, 2009).

**Voges- Proskauer Test:** Preparation of 5 % a – naphthol in ethanol and 40 % Sodium hydroxide in deionized water was done. MR-VP broth was also prepared and 5 ml dispensed in different test tubes and sterilization was done at 121 °C for 15 minutes using an autoclave. The medium was allowed to cool to room temperature. Inoculum from fresh culture media was introduced in different test tube and this was incubated together with the control at 37 °C for 48 hours. About 2.5 ml of culture was dispensed in a sterile cultures tube and 5 drops of methyl red reagent was added. The test organism was also compared with the control and colour change was observed (McDevitt, 2009).

**Nitrate Reduction Test :** Durham tubes were placed in a test tube and 15 ml nitrate broth was prepared and dispensed into same test tube. This was placed in an autocalve an sterilized at 121 °C for 15 minutes. Bacteria suspension was inoculated in the sterile broth and incubated at 37 °C for 24 hours. Formation of gas was observed for positive result. Six drops each of Nitrite reagent A and Nitrite reagent B was prepared and dispensed into the test tubes. Observation for colour changes was recorded (Tiso *et al.*, 2012).

**Coagulase Test:** Few drops of physiological saline was placed on two separate grease free slide and a loop of bacterial isolate was emulsified on the slide to make two suspensions. A drop of human plasma was collected with a sterile Pasteur pipette and mixed gently on the slides. The two slides were observed for clumping between 5-10 minutes for positive result (McAdow et al., 2012).

# **Determination of Antibiotic Susceptibility Profile**

**Preparation of McFarland Standard:** One gram (1g) anhydrous Barium chloride (Bacl<sub>2</sub>) was dispensed in 100 ml distilled water to produce 1 % Barium Chloride (Bacl<sub>2</sub>) solution. One (1) ml of concentrated (H<sub>2</sub>SO<sub>4</sub>) was also dispensed in 99 ml of distilled water to produce 1 % Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). The solutions were mixed together with 0.05 ml of 1 % Barium Chloride (BaCl<sub>2</sub>) and 9.95 ml of 1 % Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) to form McFarland standard. This is equivalent to 1.5 x 10<sup>8</sup> cfu/ml.

**Standardization of Bacterial Isolates**: A loop of 24 hours old bacterial culture was transferred into 5 ml Mueller- Hinton broth. This was placed in an incubator for 4 hours at 37 °C. Turbidity was adjusted using spectrophotometer and diluting bacterial suspention with distilled water at 625 nm. The tube was sealed and compared with McFarland standard solution (Khan *et al.*, 2019; CLSI 2009).

Antibiotic Susceptibility Test of Bacterial Isolates: 0.5 McFarland turbidity standards was used to compare culture of each bacterial isolate. Bacterial isolates (1.5 x 10<sup>8</sup> cells/ml) was seeded into each sterile Mueller- Hinton agar and were allowed to stand at room temperature (27 °C ) for 30 minutes to allow inoculated organisms to pre- diffuse in the prepared media. The disc containing antibiotics ( Septrin, Chloraphenicol, ciprofloxacin, sparfloxacin, amoxillin, gentamycin, augmentin, pefloxacin, ofloxacin and streptomycin) were carefully placed aseptically on Mueller Hinton agar plates. All plates were placed in an incubator and allowed to stand for 24 hours at 37 °C. Zone on inhibition was measured in millimeters to meet the guidelines set by the clinical standard laboratory institution (CLSI, 2017).

# Data analysis

Enumeration of bacterial count was done using colony forming unit per millimeter (cfu/ml). Pne way analysis of variance (ANOVA) and Post hoc multiple comparison test SPSS (Statistical Package for Social Science) version 20.P was used for statistical analysis.  $P \le 0.05$  was noted as statistically significant.

#### RESULTS

Total Bacterial Count of Ready to Eat Fruits and Vegetable: Microbial load of the fruits and vegetables varied with type and vendor (Table 1). Microbial load ranged from  $60.17 \pm 3.10 \times 10^4 - 158.67 \pm 6.90 \times 10^4$  cfu/ml for vendor A;  $73.33 \pm 2.36 \times 10^4 - 144.33 \pm 4.24 \times 10^4$  cfu/ml for vendor B and  $56.83 \pm 3.53 \times 10^4 - 88.50 \pm 3.10 \times 10^4$  cfu/ml for vendor C. (Table 1). Watermelon from Vendor C had the lowest microbial load ( $56.83 \pm 3.53 \times 10^4$  cfu/ml) of all the fruits and vegetables sampled, while carrot from vendor A had the highest microbial load ( $158.67 \pm 6.90 \times 10^4$  cfu/ml).

#### Table 1:

<b>Total Bacterial</b>	count of Read	v to Eat Fruits	and vegetable

	(X	t 10 <sup>4</sup> cfu/ml)	
Sample Type	Α	В	С
Watermelon	91.61	61.83	56.83
	± 2.83°	$\pm 2.60^{\mathrm{b}}$	± 3.53°
Carrot	158.67	144.33	81.33
	± 6.90°	$\pm 4.24^{b}$	± 3.77°
Pawpaw	60.17	73.33	88.50
	± 3.10°	$\pm 2.36^{\mathrm{b}}$	± 3.10°

Values are means of duplicate readings and SD of total Bacterial counts of ready to eat Fruits and vegetable. Values with different superscripts on the same row are significantly different at ( $P \le 0.05$ ). A; Vendor at the main gate of Emirs palace in Oja-Oba's market B; Vendor at the roundabout area of Oja-Oba's market

C; Vendor at the exit gate of Emirs palace in Oja-Oba's market

Total Coliforms Count of Ready to Eat Fruits and Vegetable: The bacterial count for coliforms on all samples ranges from  $7.80 \pm 1.10 \times 10^4 - 26.70 \pm 2.82 \times 10^4$  cfu/ml. Watermelon ( $7.80 \pm 1.10 \times 10^4$  cfu/ml) had the lowest coliform count compared to Carrot ( $26.70 \pm 2.82 \times 10^4$  cfu/ml) isolated from vendor A and pawpaw ( $21.50 \pm 1.65 \times 10^4$  cfu/ml) from vendor C (Table 2).

Table	2:
-------	----

	(	x 10 <sup>4</sup> cfu/ml)	
Sample Type	Α	В	С
Watermelon	17.50	07.80	12.50
	± 2.12°	$\pm 1.10^{\circ}$	$\pm 2.59^{b}$
Carrot	26.70	13.00	21.50
	± 2.82°	$\pm 1.89^{\circ}$	± 1.65 <sup>b</sup>
Pawpaw	11.83	14.12	18.12
	± 1.17°	$\pm 0.70^{b}$	± 1.64ª

Values are means of duplicate readings and SD of total coliform counts of ready to eat Fruits and vegetable. Values with different superscripts on the same row are significantly different at ( $P \le 0.05$ ). Key:

*A*; *Vendor at the main gate of Emirs palace in Oja-Oba's market B*; *Vendor at the roundabout area of Oja-Oba's market* 

C; Vendor at the exit gate of Emirs palace in Oja-Oba's market

#### Table 3:

Total *Staphylococcus* count of Ready to Eat Fruits and Vegetable

	(	(x 10 <sup>4</sup> cfu/ml)	
Sample Type	Α	В	С
Watermelon	$11.83 \pm$	$11.00 \pm$	$05.00 \pm$
	2.12ª	3.30ª	0.24 <sup>b</sup>
Carrot	21.77 ±	$13.33 \pm$	$13.33 \pm$
	3.06ª	1.41 <sup>b</sup>	5.05 <sup>b</sup>
Pawpaw	$11.67 \pm$	$17.85 \pm$	$15.00 \pm$
	0.47°	1.63ª	1.41 <sup>b</sup>

Values are means of duplicate readings and SD of total *Staphylococcus* counts of ready to eat Fruits and vegetable. Values with different superscripts on the same row are significantly different at ( $P \le 0.05$ ).

B; Vendor at the roundabout area of Oja-Oba's market

C; Vendor at the exit gate of Emirs palace in oja-Oba's market

**Morphological Characteristics of Bacterial Isolates**: Based on the morphological characteristics of bacterial isolates observed on the selective/differential media (Table 5); Isolates on MaConkey appeared Smooths, Opaque and Motile. Isolates on Eosin Methylene Blue agar appeared Pink, Mucoid, and some with green metallic sheen. Manitol Salt agar developed colonies that were yellowish and oily. A blackish substance was found on Salmonella Shigella agar with the organism appearing circular and transparent in the middle of the black substance.

**Gram Stain Reaction**: Three (3) isolates were Gram positive bacterial while nine (9) isolates were Gram negative bacteria with the characteristics colour of Purple for Gram positive and Pink for Gram negative (Table 6)

#### Table 4:

Total *Salmonella* count of Ready to Eat Fruits and Vegetable

	(x	t 10 <sup>4</sup> cfu/ml)		
Sample Type	Α	В	C 0	
Watermelon	0	0		
Carrot	9.67±1.41 <sup>b</sup>	11.67±1.41ª	$08.84 \pm 1.0$	
Pawpaw	0	0	0	
		1 65 6	1	

Values are *means* of duplicate readings and SD of total *Salmonella* counts of ready to eat Fruits and vegetable. Values with different superscripts on the same row are significantly different at ( $P \le 0.05$ ). *A*= *Vendor at the main gate of Emirs palace in Oja-Oba's market B*= *Vendor at the roundabout area of Oja-Oba's market* C= *Vendor at the exit gate of Emirs palace in Oja-Oba's market* 

Total *Staphylococcus* of Ready to Eat Fruits and Vegetable *Staphylococcus* count ranges from  $5.0 \pm 0.24 \times 10^4 - 21.17 \pm 3.06 \times 10^4$  cfu/ml for all samples. The load on watermelon and carrot for; Vendor A and B; Vendor B and C respectively are significantly the same while *Staphylococcus* count on Pawpaw samples collected from the 3 vendors are significantly different (Table 3).

Total Salmonella Count of Ready to Eat Fruits and Vegetable: There was no growth of Salmonella on all fruits sample (watermelon and pawpaw) but the vegetable (carrot) has Salmonella with a load ranging from  $11.67 \pm 1.41 - 8.84 \pm 1.18$  cfu/ml with Vendor B having the highest Salmonella count and Vendor C having the lowest count (Table 4).

Morphological Characteristics of Bacterial Isolates: Based on the morphological characteristics of bacterial isolates observed on the selective/differential media (Table 5); Isolates on MaConkey appeared Smooths, Opaque and Motile. Isolates on Eosin Methylene Blue agar appeared Pink, Mucoid, and some with green metallic sheen. Manitol Salt agar developed colonies that were yellowish and oily. A blackish substance was found on Salmonella Shigella agar with the organism appearing circular and transparent in the middle of the black substance.

#### Table 5:

Morphological Characteristics of Bacterial isolate on Selective/Differential Media

Plates	Colour	Size	Elevation	Margin	Shape	Gram's Reaction
WA-1	Opaque	Large	Flat	Lobate	Cocci	-ve
WA-2	Yellow	Small	Raised	Curled	Cocci	+ve
WA-3	Pink	Small	Umbonate	Undulate	Rod	-ve
WA-4	Green	Small	Flat	Undulate	Rod	-ve
WA-5	Opaque	Large	Flat	Lobate	Cocci	-ve
CB-1	Yellow	Small	Flat	Curled	Cocci	+ve
CB-2	Black	Small	Raised	Entire	Rod	-ve
CB-3	Pink	Small	Umbonate	Undulate	Rod	-ve
CB-4	Translucent	Medium	Raised	Undulate	Rod	-ve
CB-5	Green	Small	Flat	Undulate	Rod	-ve
PC-1	Opaque	Large	Flat	Lobate	Cocci	-ve
PC-2	Green	Small	Flat	Undulate	Rod	-ve
PC-3	Pink	Medium	Umbonate	Undulate	Rod	-ve
PC-4	Translucent	Small	Raised	Undulate	Rod	-ve
PC-5	Yellow	Small	Flat	Curled	Cocci	+ve

\*(-ve), negative; (-ve), positive; WA 1-5; Bacterial isolates on watermelon from different culture media

CB 1-5; Bacterial isolates on carrot from different culture media; PC 1-5; Bacterial isolates on pawpaw from different culture media

A; Vendor at the main gate of Emirs palace in Oja- Oba's market

Gram Stain Reaction: Three (3) isolates were Gram positive bacterial while nine (9) isolates were Gram negative bacteria with the characteristics colour of Purple for Gram positive and Pink for Gram negative (Table 6).

Biochemical reaction of the Bacterial Isolates.: Biochemical test confirmed the presence of Staphylococcus spp., Proteus spp., Enterobacter spp., Salmonella spp., Escherichia spp., and Shigella as stated on the row for probable organism (Table 7).

Antibiotics Susceptibility Test of Bacterial Isolates: The diameter of susceptibility ranges from 10.00  $\pm$  0.00 - 26.50  $\pm$ 

0.21 mm for all isolates. Ciprofloxacin, gentamycin and pefloxacin had the same diameter of zone of Inhibition (24.50  $\pm$  0.71mm) on *Escherichia* spp. The widest zone of inhibition  $(26.50 \pm 0.21 \text{ mm})$  was recorded for gentamycin against Staphylococcus spp.

Antibiotics Susceptibility Pattern of Bacterial Isolates: All antibiotics that had a zone of inhibition less than 13.00 mm were recorded as (R) resistant, values between 13.00 - 19.00 mm were recorded as (I) intermediate while value above 19.00 were recorded as (S) Susceptible (Table 8).

#### Table 6:

Isolates	OX	CA	СО	IND	CIT	MR	NIT	VP	Probable Organism
WA-1	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	Proteus Spp.
WA-2	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	Staphylococcus spp.
WA-3	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	Enterobacter spp.
WA-4	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Escherichia spp.
WA-5	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	Proteus spp.
CB-1	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	Staphylococcus spp.
CB-2	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Salmonella spp.
CB-3	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	Enterobacter spp.
CB-4	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	<i>Shigella</i> spp.
CB-5	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Escherichia spp.
PC-1	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	Proteus Spp.
PC-2	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Escherichia spp.
PC-3	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	Enterobacter spp.
PC-4	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Shigella spp.
PC-5	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	Salmonella spp.

\*(+ve), positive; (-ve), negative; IND, indole; OX, oxidase; CA, catalase; CO, coagulase; CIT, citrate; MR, methyl red; NIT, nitrate; VP, vogeproskauer.

WA 1-5: Bacterial isolates on watermelon

CB 1-5: Bacterial isolates on carrot

PC 1-5; Bacterial isolates on pawpaw

Isolates	SXT	СН	SP	СРХ	AM	AU	CN	PEF	OFX	S
Proteus	$10.25 \pm$	$12.50 \pm$	$19.50 \pm$	$19.50 \pm$	$14.50 \pm$	$15.50 \pm$	$19.50 \pm$	$22.50 \pm$	$14.50 \pm$	$10.00 \pm$
	0.21	0.42	0.14	0.10	0.00	0.71	0.14	0.71	0.14	0.00
Entebobacter	$10.00 \pm$	$15.50 \pm$	$10.00 \pm$	$10.00 \pm$	$10.00 \pm$					
	0.00	0.71	0.71	0.00	0.00	0.00	0.71	1.41	1.41	0.00
Escherichia	$15.50 \pm$	$10.00 \pm$	$16.50 \pm$	$24.50 \pm$	$10.00 \pm$	$10.50 \pm$	$24.50 \pm$	$24.50 \pm$	$23.50 \pm$	$11.50 \pm$
	0.71	0.71	0.00	0.71	0.00	0.71	0.71	0.00	0.71	0.71
Salmonella	$11.50 \pm$	$10.00 \pm$	$12.50 \pm$	$15.75 \pm$	$11.50 \pm$	$10.00 \pm$	$15.50 \pm$	$17.50 \pm$	$16.5 \pm$	$13.50 \pm$
	0.70	0.71	0.71	0.35	0.70	0.71	0.71	0.00	0.00	0.71
Shigella	$10.00 \pm$	$10.25 \pm$	$10.00 \pm$	$18.00 \pm$	$10.00 \pm$	$10.00 \pm$	$17.00 \pm$	$16.00 \pm$	$13.00 \pm$	$10.00 \pm$
	0.70	0.35	0.00	0.70	0.00	0.00	0.00	0.70	1.41	0.00
Staphylococcus	$10.00 \pm$	$10.00 \pm$	$10.00 \pm$	$24.50 \pm$	$12.00 \pm$	$16.00 \pm$	$26.50 \pm$	$23.50 \pm$	$10.00 \pm$	$15.50 \pm$
	0.71	0.71	0.71	0.71	0.00	0.00	0.21	0.00	0.00	0.71

Values are means of duplicate reading and  $\pm$  S.D of zone of inhibition against isolates.

Keys: SXT, septrin; CH, chloramphenicol; SP, sparfloxacin; CPX, ciprofloxacin; AM, amoxicillin; AU, augmentin; CN, gentamycin; PEF, pefloxacin; OFX, ofloxacin; S, streptomycin.

Table 7:

antibiotics bensitivit	y i allein ag	sumst ber	Letted Daei		idy to Lat	Truns and	vegetable			
Isolates	SXT	СН	SP	СРХ	AM	AU	CN	PEF	OFX	S
Proteus	R	R	R	R	R	R	R	S	Ι	R
Entebobacter	R	R	R	R	R	R	S	R	R	R
Escherichia	S	R	Ι	S	R	R	S	S	S	R
Salmonella	R	R	R	R	R	R	R	R	S	S
Shigella	R	R	R	S	R	R	R	R	R	R
Staphylococcus	R	R	R	S	R	Ι	S	S	R	R

Antibiotics Sensitivity Pattern against Selected Bacteria on Ready to Eat Fruits and Vegetable

Key: R-Resistance, S-Susceptible, I-Intermediate; SXT, septrin; CH, chloramphenicol; SP, sparfloxacin; CPX, ciprofloxacin; AM, amoxicillin; AU, augmentin; CN, gentamycin; PEF, pefloxacin; OFX, ofloxacin; S, streptomycin.

# DISCUSSION

Table 8:

Results from this study show the presence of harmful bacteria in fruits and vegetable samples purchased from Oja-Oba market, Ilorin. The isolated organisms were; *Escherichia* spp., *Enterobacter* spp., *Staphylococcus* spp., *Salmonella* spp., *Proteus* spp., and *Shigella* spp. The highest level of bacterial load was recorded on carrot (Table 1). Cross contamination of fresh produce could have been from the vendor or the environment since the operating premises is usually kept unclean. The previous study of Akusu *et al.*, (2016) on vegetable salads from street foods among different vendors in Port Harcourt metropolis in Nigeria agrees with the present study as high bacterial load was observed in some of the selected foods.

Increase in the number of human infections and outbreaks is a resultant effect of the high rate in consumption of fruits and vegetables (Mashak et al., 2015) as most pathogenic or opportunistic bacteria inhabit them (Berg et al., 2014). Contamination of fruits and vegetables by spoilage organism or harmful bacteria usually occur at any stage of production to the consumer (Berg et al., 2014). Fruits and vegetables have there microflora which are often yeast, molds and spoilage bacteria, it was also discovered that they can harbor harmful bacteria such as Escherichia coli, Salmonella, Shigella, Listeria, *Campylobacter*, Monocytogenes, Yersinia enterocolitica, Clostridium botulinum, Bacillus cereaus as well as parasite (Mritunjay and Kumar, 2015). Microbes that are non-pathogenic are found to increase the rate of spoilage hereby diminishing the quality of the produce and reduction in market value. Since fruits and vegetables harbours microorganisms they also help in spreading microbes from one area to other areas food is being prepared (Altieri and Nicholls, 2017). The presence of coliforms on all samples (Table 2) could be a result of feacal contamination or an indication of poor sanitary of the vendors (Oje et al., 2018). The presence of Escherichia, Staphylococcus, Salmonella, Enterobacter and Shigella is of great concern in public health since most of these microorganisms are virulent. Olawale et al., (2015) reported different prevalence of virulent genes in Enterococcus feacalis isolated from ready to eat foods. Foodborne illnesses are a growing public health distress, social disturbance, avoidable economic burden and preventable death. One of the normal flora of the human and animal intestine is Esherichia coli often known as enteric bacteria is one of the common cause of foodborne illness in the world (Sharff, 2012). Salmonella spp. Should not be found in 25 g of ready to eat fruits and vegetable meant for human consumption and therefore must be rejected (Food and Drugs Board, 2013; Abakari and Cobbina, 2018). The presence of Salmonella on the carrot sample depicts that the vegetable is unfit for human consumption going by the guidelines. Shigella spp. Isolated on both watermelon and carrot samples is unsatisfactory for human consumption according to health protection agency, 2019 that states that food containing Shigella in about 25 g of sample is unsafe for human consumption (Abakari and Cobbina, 2018). The fruits and vegetable sample contaminated with *Shigella* spp. could be as a result of improper hygiene practices by vendors in Oja-Oba market, Ilorin. The level of hygiene practices can influence the total number of bacterial load on fruits and vegetables. The source where the fruits and vegetable are gotten could have influenced the high number of bacterial load in selected samples. Several factors in each collection point could have contributed to contamination on samples. Data analysis of mean bacterial count isolated reveals a significant difference across vendors at P value  $\leq 0.05$ . Bakobie *et al.*, 2017 reported that there was no significance difference in pathogenic bacteria countdone on fruits and vegetable sample from different location in central part of Durban. This study has identified the presence of Enterobacter, Escherichia, Salmonella, Proteus, Shigella and Staphylococcus on selected fruits and vegetable sample obtained from the central market Oja-Oba, Ilorin. The isolated bacterial subjected to ten (10) antibiotics test shows high multiple resistance which is of great concern (Table 8). The selected isolated bacterial from this study were resistance to amoxilin and chloraphenicol these is similar to previous study by Getie et al., 2015 who carried out a study on anticlimactic susceptibility pattern of certain enteric bacteria among food handlers in Gonda town, Ethiopia. The effective antibiotics include Ciprofloxacin, gentamycin and pefloxacin which can be used to treat infections from Escherichia. The widest zone of inhibition was recorded for gentamycin against Staphylococcus spp.

In conclusion, the present study has shown that ready-toeat fruits and vegetable sold by street vendors in Oja-Oba market are not safe for human consumption and consumers are at health risk in terms of microbial quality. Contamination from farms or production area, Improper food handling while processing, non-hygienic practices while packaging and environmental conditions are major factors responsible for high microbial load on fruits and vegetables. This study shows that there is an urgent need for regulation agency to vet food vendors in other to promote improvement in quality standards and food safety of ready-to-eat foods in Oja-Oba market, Ilorin metropolis. It is recommended that food and drug authority should ensure that the street vendors are educated on good and proper hygiene while processing fresh produce for human consumption and enforce strict compliance.

# REFERENCES

Abdolmaleki, Z., Mashak, Z. and Dehkordi, S. (2019). Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant Staphylococcus aureus hospital strains isolated from cockroaches. Antimicrobial Resistance and Infection Control, 8 (54): 2-14. Adegoke, A. A. and Okoh, I. A. (2013). Staphylococcus species and emerging traits in their commensal subgroup: A Call to Arms. Journal of Pure and Applied Microbiology, 7(4): 3281-3291.

Adegoke, A.A. and Okoh, A.I. (2015). Antibiogram of *Stenotrophomonas maltophilia* isolated from Nkonkobe Municipality, Eastern Cape Province, South Africa. *Jundishapur Journal of Microbiology*. 8(1): e13975.

Adegoke, A.A. and Okoh, A.I. (2014). Species diversity and antibiotic resistance properties of Staphylococcus of farm animal origin in Nkonkobe Municipality. *Folia Microbiology*, 59:133-140.

Adegoke, A.A., Faleye, A.C., Singh, G. and Stenström, T.A. (2016). Antibiotic Resistant Superbugs: Assessment of the Interrelationship of Occurrence in Clinical Settings and Environmental Niches. Molecules, 22(1): 29;

Akinduti, P. A., Ejilide, O., Motayo, B. O and Adeyokinu, A. F.(2012). Emerging multi-drug resistant. AmPC betalactamase and carbepenase enteric isolates in Abeokuta, Nigeria. *Nature and Science*, 10(7): 70-74.

**Becker, K., Heilmann, C. and Peters, G. (2014).** Coagulase-Negative *Staphylococci. Clinical Microbiology Reviews,* 27: 870–926.

**Ben, M., Cark, Y. and Rik, G. (2017).** *Staphylococcus aureus* isolated from wastewater treatment plants in Tunisia: occurrence of human and animal associated lineages. *Journal of Water Health*, 65: 23-36.

**Cassat, J., Lee, C. and Smeltzer, M. (2007).** Investigation of biofilm formation in clinical isolates of *Staphylococcus aureus. Review of Methods in Molecular Biology*, 391:127–134.

**Clinical and Laboratory Standard-CLSI. (2015).** Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. In: CLSI document M100- S25. Wayne.

**Cramton, S., Gerke, C., Schnell, N., Nichols, W. and Götz, F. (1999).** The intercellular adhesion (ICA) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infection and Immunity*, 67:5427–33.

Dehkordi, S., Gandomi, H., Akhondzadeh-Basti, A., Misaghi, A. and Rahimi, E. (2017). Phenotypic andgenotypic characterization of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolated from hospital food. *Antimicrobial Resistance and Infection Control*, 6:104-107.

**Donkor, E. S. (2019).** Nosocomial Pathogens: An In-Depth Analysis of the Vectorial Potential of Cockroaches.

Tropical Medicine and Infectious Disease, 4: 14-24.

**Evans, J. and Wittler, M. (2019).** *Amoxicillin Clavulanate.* StatPearls Publishing, Treasure Island.

Gary, M and Durben, L. (2004). Medical and vertinary entomology. *Medical and Veterinary Practices News*, 597: 204 – 285.

**Gray, M. and Durden, L. (2004).** Medical and Veterinary entomology. *Medical and veterinary pratices news*, 597:04 – 85.

**Gehad, T. E and Emman, T. E (2011).** The role of cockroaches and flies in mechanical transmission of medical important parasites. *Journal of Entomology and Nematology*, 3(7):98-104.

Harwood, R. and James, M. (1979). Entomology in human and animal health. 7th Edition. New York, Macmillan Publishing.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stately, J. T. and Williams, S. T. (1994). *St. Bergey's Manual of Determinative Bacteriology* (9th Edition). Baltimore, Williams and Wilkins. 787p

**Ifeanyi, O. (2015).** Microbiology of Cockroaches – A Public Health Concern. *International Journal of Scientific Research*, 4 (4): 22-34.

**Melton, L. (2012).** Mechanical vectors of bacterial GI diseases. *Acta tropica*, 78: 3 – 17.

Menasria, T., Moussa, F., El-Hamza, S., Tine, S., Megri, R. and Chenchouni, H. (2014). Bacterial load of German cockroach (*Blattella germanica*) found in hospital environment. *Pathogens and Global Health*, 108: 141–147. Pai, H., Chen, W. and Peng, C. (2005). Isolation of bacteria with antibiotic resistance from household cockroaches

(Periplaneta americana and Blattella germanica). Acta Tropica, 93: 259–265.

**Pollianna, S., Souza, G., Campos, B., Yatsuda, R. and Marques, L. (2014).** Isolation, pathogenicity and disinfection of Staphylococcus aureus carried by insects in two public hospitals of Vitória da Conquista, Bahia, Brazil. *The Brazilian Journal of Infectious Diseases*, 18(2) :129–136.

**Regenthal, P., Hansen, J.S., André, I. and Lindkvist-Petersson, K. (2017).** Thermal stability and structural changes in bacterial toxins responsible for food poisoning. PLoS One, 12(2):e0172445.

**Tsutsuura, S. and Murata, M. (2017).** Temperature dependence of staphylococcal enterotoxin A production by *Staphylococcus aureus. Nihon Rinsho.* 70(8):1323-1328.

Valles, S. M., Koehler, P. G. and Brenner, R. J. (1999). Comparative insecticide susceptibility and detoxification enzyme activities among pestiferous btattodea. *Compartive Biochemistry and PhysiologyToxicology*.124(3): 227–232