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Antibiogram of *Salmonella* Species Isolated from Tomatoes (*Solanum lycopersicum*)

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ABSTRACT

Tomatoes (*Solanum lycopersicum*) are globally consumed vegetables exposed to microbial contamination. The objective of the study was to determine the presence of *Salmonella* species on tomato fruits in three Local Government Areas (LGAs) of Rivers State such as Tai, Obio/Akpor and Port Harcourt. Five (5) different market were sampled in each LGAs. Bacteriological analysis of 90 samples of tomatoes (45 healthy tomato (HT) + 45 unhealthy tomato (UHT) collected from different sources was carried out. Analysis was by culturing the tomatoes on different laboratory media. Results indicated that the total heterotrophic bacterial (THB) counts in HT and UHT ranged from $2.01\pm1.92x10^7$ – 6.85 ± 1.17 $x10^9$ (CFU/g) while the total *Salmonella* Counts (TSC) $0.37\pm0.14x10^2$ - 7.49 ± 0.92 $x10^2$ (CFU/g) which indicated a significant difference ($P\leq0.05$) for THB and no significant difference ($P\geq0.05$) for TSC. Results indicated that Tai had the highest THB counts while Obio/Akpor had the highestTSC. Following the conventional methods of bacterial identification, using sugar fermentation and biochemical tests, twenty-two (22) isolates of bacteria were identified out of which five (5) species of *Salmonella* were genomically identified.The results of the susceptibility pattern of *Salmonella species* indicated that *Salmonella enterica subsp. enterica strain* ON832663 and *Salmonella enterica subsp. enteric* AB681241 were susceptible to Ofloxacin and Levofloxacin (100%) respectively while *Salmonella bongori strain* MZ959600 and *Salmonella enteric strain* OP745459 showed a decreasing trend of resistance in the order; Ampiclox and Ceftriazone- Sulbactam (92.9%) respectively. This result highlights the fact that tomatoes in the three Local Government Areas are not safe microbiologically for human consumption without additional treatment such as proper washing, boiling or disinfection and this could lead to outbreak of food borne diseases.

Keywords: Unhealthy tomato fruit, *Salmonella* species, *Salmonella enterica*, antibiotic resistance, SHV gene.

Introduction

Tomato (*Solanum lycopersicum*) is the third most cultivated vegetable crop globally and consumed as food, nutrient supplement, flavouring ingredient, medicine, lowers the risk of a variety of cancers such as prostate and cervical cancers and cardiovascular disorders, detoxificant, human system cleanser (blood and urinary tract) and enhances fertility in men (Bello *et al.*, 2016).

It is a major horticultural crop with an estimated total world production of 152.9-173 million tonnes and contributes to the economy of many nations (Abiso *et al.*, 2015). Nigeria is the second largest producer of tomato fruits in Africa and 13th largest in the world (Taofiq, 2017).

The production of tomatoes in Nigeria in 2010 was 1.8 million metric tonnes whereas national demand is about 2-3 million metric tonnes annually, this deficit gap resulted in importation of 105,000 metric tonnes of tomato paste valued over ₦16 billion between 2009 and 2010 making Nigeria one of the primary importers of tomato globally (Adegbola *et al.*, 2012). Tomatoes contribute to a healthy, well-balanced diet as they are rich in minerals, vitamins, essential amino acids, sugars, dietary fibres, vitamin B and C, iron and phosphorus (Toor, 2006; Babalola *et al.*, 2010; El-Dengawy *et al.*, 2016).

On the farm, produce is exposed to *Salmonella* by contact with wildlife, contaminated irrigation water, untreated manure.

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Poor hygiene by fieldworkers, use of mobile toilets and hand-washing stations increase the risk of pathogen dissemination at pre-harvest and during harvest. After harvest, contamination of produce is mainly due to poor hygienic practices. Bennett *et al*. (2015) noted that tomatoes specifically were implicated in 15 multi-state outbreaks of salmonellosis between 1990 and 2010. Traceback analysis suggested that contamination happened during the production or processing stages. Devleesschauwer *et al*., (2017) noted that although salmonellosis outbreaks due to fruits and vegetables have been well documented, their occurrence, however, remains sporadic. Moreover, Devleesschauwer *et al*. (2017) also stated that for outbreaks involving fruits and vegetables to occur, a multitude of factors must come together. These factors include the presence of vectors, level of crop maturity, physiological defects, presence of native biota that may inhibit or promote human pathogens, type of irrigation practiced, etc. The role of environmental conditions and farm practices is also essential in determining the factors that make plants susceptible to *Salmonella* proliferation both pre and post-harvest. The study carried out by Devleesschauwer *et al.,* (2017) confirmed that harvesting tomatoes when still green significantly reduces *Salmonella* infestation, as does harvesting after a period of high humidity. Pre-harvest application of copper, iron, potassium, nitrogen or foliar sprays did not affect post-harvest contamination (Jain *et al*., 2019).

Traditionally, plants are not recognized as hosts for human pathogens such as *Salmonella species* but in the last few decades, the niches for these organisms have changed. *Salmonella species* produces periplasmic enzymes with the ability to break plant surface barriers. However, the penetration of these enzymes into plant systems is dependent on pectin and polygalacturonate processing (level of ripening) and physiological wounds (Devleesschauwer *et al*., 2017). Members of the Enterobacteriaceae family are capable of penetrating the stomata of plant leaves, hydratodes and roots. Plants contaminated pre- or post-harvest do not exhibit signs of spoilage while the organisms contaminate the produce whether pre-harvest or post-harvest. According to the World Health Organization (WHO, 2019), one of the agents triggering foodborne disease is *Salmonella* spp., which causes salmonellosis disease with a high morbidity and mortality rate in industrialized and developing countries.

Salmonella species are one of the most common causes of food and water-borne gastroenteritis in humans, which remains an important health problem Worldwide. According to World Health Organization (WHO) estimates, there are about 16 million new cases and 600,000 deaths from typhoid fever each year worldwide (WHO, 2019).

Salmonella spp is a gram-negative rod with peritrichous flagella. Moreover, *Salmonella* is a chemoorganotrophic organism and a facultative anaerobe, capable of using both fermentative and respiratory metabolic pathways. These capabilities cause contamination in food-production chains, since *Salmonella* spp. can survive for a long period of time under food-stored conditions. *Salmonella* spp can be categorized into typhoidal and non-typhoidal varieties based on the disease it causes (Park *et al.*, 2013; McFarland *et al.*, 2019).

For many years, the phenotypic trait of MDR was widely distributed among *S. typhi* and, at a lower rate, among *S. paratyphi* (Devleesschauwer *et al.*, 2017). Africa and Asia are two continents with a high isolation frequency of *S. typhi* displaying MDR phenotype. In a surveillance study conducted in five Asian countries, India, Pakistan and Vietnam had higher rates of MDR isolates of *S.* Typhi than Indonesia and China. Other reports present similar data, with a high rate of MDR isolates of *S*. Typhi in Pakistan, India, Nepal and Vietnam, while in China, Indonesia and Laos the incidence rate of MDR *S. typhi is* relatively low (Crump *et al.*, 2004).

The emergence of antimicrobial resistance in *Salmonella* strains is a serious health problem worldwide (Connor *et al.*, 2010). In the early 1960s, the first incidence of *Salmonella* resistance to a single antibiotic, namely chloramphenicol was reported (Akbar *et al.*, 2015). Since then, the frequency of isolation of *Salmonella* strains with resistance towards one or more antimicrobial agents has increased in many countries, including the USA, UK and Saudi Arabia (Nguyen *et al.*, 2016). Antimicrobial agents such as ampicillin, chloramphenicol and trimethoprim–sulfamethoxazole are used as the traditional first line treatments for *Salmonella* infections. *Salmonella* spp. resistant towards these agents are referred to as multi-drug resistant (MDR).

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Tomatoes could be contaminated with *Salmonella species* on farms, during processing and food preparation. Although foodborne outbreaks related to tomatoes do not appear to be increasing, the risk remains (Gurtler *et al.*, 2018). Furthermore, the proportion of all foodborne outbreaks attributable to fresh produce has been increasing (Bennett *et al.*, 2018).

The aim of this present study is to determine the antibiogram of *Salmonella* species isolated from healthy and unhealthy tomato fruits in Three Local Government Areas in Rivers State, Nigeria as to ascertain the health risks associated with the consumption of unhealthy tomato fruits.

Materials and Methods

Description of the Study Area

The study area was Rivers State in Nigeria. Markets in three Local Governments Areas viz; Obio/Akpor LGA, Port Harcourt LGA and Tai LGA were chosen from the study area based on the high population of people living in these areas. Five (5) markets were selected from each of these LGAs making a total of fifteen (15) markets used for this present study. Markets in Obio/Akpor LGA were: Big Tree Market (4^o45'42.3"N7^o01'19.3E), Rumuokoro Market ($4^0$52'027^{0}N$6^0$59'39.8"E), Nkpor Market ($4^0$52'1"N $6^0$59'40"E), Omoku Market ($4^0$52'1"N $6^0$59'39"E), and Choba Market (4^o54'17.4"N6^o54'14.1"E).

Markets in Port Harcourt LGA were: Mile3 Market (4^o47'3"N6^o58'59.5"E), Mile1 Market ($4^0$51'41'N $7^0$1'47" E), Fruit Garden Market ($4^0$51'52" N $7^0$1'52" E), Emenike Market ($4^0$51'46" N $7^0$1'51" E), and Sangana Market ($4^0$51'46" N $7^0$1'51" E) while markets in Tai LGA were: Nonwa Market ($4^0$42'59.99'N$7^0$17'60.00'E), Kira Market ($4^0$43'0'N $7^0$18'0'E), Kpite Market ($4^0$42'59.9"N"$7^0$17'60E), Korokoro Market (4^0 42' 59.99" N 7^0 17' 60.00") and Gbam Market (4^0 73' 58.8" N 7^0 22' 85.8" E), with five (5) sampling market locations in each of the three Local Government Areas.

A total of 30 samples of tomatoes were collected from each market of the three LGAs and sampling was carried out three (3) times in each of the three markets.

Study Design and Sample Collection

This study was a descriptive cross-sectional study. Thirty (30) samples of tomato fruits from each of the three LGAs) tomato samples were bought from tomato sellers in different market sampling locations in the three Local Government Areas: (Obio/Akpor, Port Harcourt and Tai LGAs); and five (5) sampling points from each location. The tomatoes were labeled properly, put in sterile plastic rubbers and transported aseptically to the Laboratory of Department of Microbiology, Rivers State University for bacteriological analysis. A total number of ninety (90) tomatoes were purchased during the study period.

Sample Preparation

Preparation of the homogenate was done by weighing 25g of tomato sample and homogenized in 225ml of diluent (normal saline).

Microbiological Analysis

Enumeration and Isolation of Bacteria

Serial tenfold dilution was done on the homogenized sample of tomato (25g in 225ml of normal saline) with dilution factor from10^{-1} to 10^{-6}. Aliquot (0.1ml) of appropriate dilutions were spread plated in triplicates onto Nutrient Agar (NA) which was used for the total heterotrophic bacteria counts and *Salmonella-Shigella* Agar (SSA) for total *Salmonella* counts. The plates (NA & SSA) were incubated at 37^{o}C for 24 hours. The colonies formed on the plates were counted and described morphologically. The colonies formed on *Salmonella-Shigella* Agar plates were used for the enumeration of the population of *Salmonella* spp. Colonies formed on Nutrient Agar were used to estimate total heterotrophic bacterial counts (THBC). Representative distinct colonies were purified by sub-culturing on freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hours to obtain pure cultures. Bacteria colonies that grew on the respective plates were enumerated as described by Prescott *et al* (2005). Bacterial colonies were counted and the mean expressed as colony forming unit per milliliter (CFU/ml) using the formula below:

$$\text{CFU/g} = \frac{\text{number of colonies}}{\textit{Dillution x volume plated}}$$

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Pure Culture Isolation and Preservation

The discrete bacterial colonies that grew on the respective media were subcultured using streak plate method onto freshly prepared nutrient agar and incubated at 37^{0}C for 24 hours in order to obtain pure cultures. The pure bacteria cultures were then maintained according to the method adopted by Amadi *et al*. (2014) using ten percent (v/v) glycerol suspension and stored at -4^{o}C as a cryopreservative agent to prevent the damage of the pure cultures for further analysis.

Identification of *Salmonella* species

Colonial/morphological characteristics and Biochemical tests were conducted on the pure isolates for identification of *Salmonella* spp. Suspected *Salmonella* spp isolates were cultured on *Salmonella-Shigella* Agar to observe for the growth of *Salmonella* spp. with a black center colony on the media which is the characteristics of the organism. Gram Staining, Motility test and Biochemical tests such as Triple sugar iron Test (TSI), Indole, Methyl red, Voges proskaur, Glucose, Lactose, Mannose, Sucrose and Citrate Utilization test were carried out to confirm *Salmonella* spp (Moreb *et al*., 2017). Molecular technique (PCR) was used to confirm the identities of the isolate to the species level (Cheesbrough, 2005).

Results

The results of the bacterial Counts of healthy and unhealthy tomatoes from various locations sampled in Obio-Akpor LGA are presented in Table 1. The mean values of total heterotrophic bacterial counts ranged from 1.02 $\pm$ 0.02x10^{7}CFU/g to 1.87±0.09x10^9 CFU/g .

The results of the bacterial Counts of healthy and unhealthy tomatoes from various locations sampled in Port Harcourt LGA are presented in Table 2. The mean values of total heterotrophic bacterial counts ranged from 1.16±0.14x10 ^{7}CFU/g to 2.05±0.35x10^{9}CFU/g while Tai LGA ranged from 1.66±0.58x10^{7}CFU/g to 1.58±0.09^{9}CFU/g.

Table 1: Population means of Total heterotrophic bacteria of healthy and unhealthy tomato from Obio-Akpor LGA Markets

Sample	Obio-Akpor LGA Markets (Healthy Tomatoes) ($x10^7$ CFU/g)					Obio-Akpor LGA Markets (Unhealthy Tomatoes) ($x10^9$ CFU/g)				
	Big Tree	R/okoro	Nkpor	Omoku	Choba	Big Tree	R/okoro	Nkpor	Omoku	Choba
1	10.3±0.15	10.3±0.58	7.67±1.53	6.00±1.00	7.67±2.52	0.16±0.18	1.77±0.11	1.35±0.14	1.35±0.45	1.46±0.16
2	6.33±1.53	93.3±2.08	6.67±1.53	6.33±2.08	8.33±1.53	1.68±0.26	1.93±0.66	1.30±0.56	1.54±0.71	1.41±0.21
3	6.00±1.00	1.00±0.20	2.33±0.58	7.67±1.53	6.67±0.58	1.60±0.62	1.90±0.20	1.10±0.84	1.33±0.50	1.29±0.32
Mean	4.50±3.00	1.02±0.02	5.56±2.84	6.67±0.88	7.56±0.84	1.63±0.05	1.87±0.09	1.25±0.13	1.41±0.12	1.34±0.09

Table 2: Population means of Total heterotrophic bacteria of healthy and unhealthy tomato from Port Harcourt LGA Markets

Sample	Port Harcourt LGA Markets (Healthy Tomatoes) ($x10^7$ CFU/g)					Port Harcourt LGA Markets (Unhealthy Tomatoes) ($x10^9$ CFU/g)				
	Mile 3	Mile1	Fruit Garden	Emenike	Sangana	Mile 3	Mile1	Fruit Garden	Emenike	Sangana
1	10.0±0.20	8.67±1.53	7.33±3.21	3.67±0.58	5.00±2.00	1.80±0.17	1.93±0.30	1.80±0.36	1.42±0.72	1.56±0.62
2	12.0±0.15	8.33±1.20	6.67±1.20	5.67±1.12	5.67±1.20	1.90±0.60	1.80±0.20	1.73±0.15	1.53±0.80	1.62±0.50
3	1.27±0.25	6.33±1.53	8.33±1.53	6.67±1.53	6.33±0.58	2.45±0.50	2.00±0.10	2.01±0.15	1.71±0.15	1.60±0.25
Mean	1.16±0.14	7.78±1.22	7.44±0.84	5.34±1.53	5.67±0.67	2.05±0.35	2.00±0.10	1.85±0.15	1.55±0.15	1.60±0.03

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Table 3: Population means of Total heterotrophic bacteria of healthy and unhealthy tomato from Tai LGA Markets

Sample	Tai LGA Markets (Healthy Tomatoes) ($x10^7$ CFU/g)					Tai LGA Markets (Unhealthy Tomatoes) ($x10^9$ CFU/g)				
	Nonwa	Kira	Kpite	Korokoro	Gbam	Nonwa	Kira	Kpite	Korokoro	Gbam
1	6.67±1.53	6.00±1.00	4.33±0.58	3.33±0.58	1.33±0.58	1.64±0.20	1.41±0.36	1.49±0.15	1.21±0.21	1.93±0.15
2	4.00±1.00	5.00±1.00	3.33.0.58	4.67±0.58	1.33±0.58	1.48±0.25	1.32±0.15	1.40±0.15	1.01±0.15	1.21±0.15
3	0.10±0.10	7.33±1.15	5.67±1.15	4.00±0.00	2.33±0.58	1.61±0.10	1.50±0.12	1.30±0.20	1.20±0.21	1.05±0.50
Mean	3.90±2.84	6.11±1.17	4.44±1.17	4.00±0.67	1.66±0.58	1.58±0.09	1.41±0.09	1.40±0.10	1.14±0.11	1.40±0.47

Results of the Total *Salmonella* count in both healthy and unhealthy tomatoes in Obio-Akpor LGA are presented in table 4. The results ranged from 0.56±0.10x10^2CFU/g to 2.81±2.00x10^2CFU/g.

Results of the Total *Salmonella* count in both healthy and unhealthy tomatoes in Port Harcourt LGA are presented in Table 5. The results ranged from 0.07±0.12x10^2CFU/g to 1.28±0.69x10^2CFU/g.

While Table 6 shows Total *Salmonella* count of Tai LGA ranged from 0.50±0.47x10^2CFU/g to 1.30±0.34 x10^2CFU/g.

Table 4: Population means of *Salmonella* of healthy and unhealthy tomato from Obio-Akpor LGA Markets

Sample	Obio-Akpor LGA Markets (Healthy Tomatoes) ($x10^2$ CFU/g)					Obio-Akpor LGA Markets (Unhealthy Tomatoes) ($x10^2$ CFU/g)				
	Big Tree	R/Okoro	Nkpor	Omoku	Choba	Big Tree	R/Okoro	Nkpor	Omoku	Choba
1	5.00±0.60	0.83±0.30	1.67±0.33	1.67±0.60	1.67±0.33	1.50±0.60	3.17±0.28	2.80±0.33	1.22±0.21	2.33±0.24
2	1.10±0.30	0±0	0±0	0.50±0.23	1.50±0.23	1.00±0.41	0.70±0.55	0.52±0.41	0.40±0.30	1.43±0.83
3	2.33±0.41	1.00±0.24	0±0	0±0	0.20±0.0	1.40±0.50	1.67±0.34	1.13±0.30	1.03±0.42	0.83±0.43
Mean	2.81±2.00	0.61±0.54	0.56±0.10	0.72±0.07	1.12±0.80	1.3±0.26	1.85±1.24	1.48±1.18	0.88±0.43	1.53±0.75

Table 5: Population means of *Salmonella* of healthy and unhealthy tomato from Port Harcourt LGA Markets

Sample	Port Harcourt LGA Markets (Healthy Tomatoes) ($x10^2$ CFU/g)					Port Harcourt LGA Markets (Unhealthy Tomatoes) ($x10^2$ CFU/g)				
	Mile 3	Mile1	Fruit Garden	Emenike	Sangana	Mile 3	Mile1	Fruit Garden	Emenike	Sangana
1	0.32±0.30	0.20±0.50	0±0	0.20±0.50	0±0	0.67±0.10	2.07±0.60	1.20±0.60	1.67±0.60	1.21±0.00
2	0±0	0.00±0.00	0±0	0±0	0.52±0.13	1.00±0.20	0.77±0.30	1.00±0.00	0.67±0.60	1.05±0.60
3	0.44±0.50	0±0	0.21±0.0	0.25±0.10	0.50±0.10	1.00±0.10	1.00±0.10	1.00±0.00	1.00±0.05	1.00±0.10
Mean	0.25±0.23	0.07±0.12	0.77±0.12	0.15±0.01	0.34±0.30	1.00±0.19	1.28±0.69	1.07±0.11	1.11±0.51	1.09±0.11

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Table 6: Population means of *Salmonella* (CFU/g) of healthy and unhealthy tomato from Tai LGA Markets

Sample	Tai LGA Markets (Healthy Tomatoes) ($x10^2$ CFU/g)					Tai LGA Markets (Unhealthy Tomatoes) ($x10^2$ CFU/g)				
	Nonwa	Kira	Kpite	Korokoro	Gbam	Nonwa	Kira	Kpite	Korokoro	Gbam
1	1.00±0.10	0.42±0.10	1.20±0.10	0.60±0.05	0.66±0.00	1.20±0.00	0.67±0.00	1.00±0.20	1.17±0.20	1.22±0.60
2	0.67±0.60	0.07±0.00	0.67±0.30	1.21±0.10	1.00±0.00	0.67±0.10	0.87±0.30	1.07±0.40	1.00±0.30	1.67±0.10
3	1.02±0.00	1.00±0.03	1.00±0.00	0.80±0.00	1.42±0.07	0.97±0.30	1.66±0.50	1.67±0.40	1.00±0.60	1.00±0.50
Mean	0.90±0.20	0.50±0.47	1.05±0.27	0.87±0.31	1.07±0.00	0.95±0.27	1.07±0.52	1.25±0.37	1.06±0.10	1.30±0.34

The results of the bacterial Counts of unhealthy tomato from various locations sampled are presented in Table 7. The result of analysis showed that the mean total heterotrophic bacterial counts ranged from 5.49±0.76 $x10^9$ CFU/g - 6.85±1.17$x10^9$CFU/g and Tai had the highest bacterial counts while the least bacterial counts was in Obio/Akpor. There was no significant difference ($p \leq 0.05$) in the total heterotrophic bacterial count between the locations sampled.

Result of the Total *Salmonella* count ranged from 5.18±0.48 $x10^2$CFU/g - 7.49±0.92 $x10^2$CFU/g. The results revealed that Obio/Akpor had the highest bacterial contamination while Tai had the least bacterial contamination. There was no significant difference ($p \leq 0.05$) in the total *Salmonella* counts between the locations sampled. These results agree with the work of Afreen *et al.*, 2019.

Table 7: Population means of bacteria of the Healthy tomato from the three Local Government Areas

Locations (LGAs)	Healthy tomato		Unhealthy tomato	
	Total Heterotrophic Bacteria Count ($\times 10^7$ CFU/g)	Total *Salmonella* Count ($\times 10^2$ CFU/g)	Total Heterotrophic Bacteria Count ($\times 10^9$ CFU/g)	Total *Salmonella* Count ($\times 10^2$ CFU/g)
Obio/Akpor	2.01±1.92[a]	0.41±0.63[b]	5.49±0.76[a]	7.49±0.92[a]
Port Harcourt	2.69±1.99[a]	0.00±0.00[a]	6.54±0.92[a]	5.18±0.48[a]
Tai	14.36±21.86[b]	0.37±0.14[b]	6.85±1.17[a]	6.00±0.39[a]
P-value	0.026	0.054	0.915	0.630

*Means with different alphabet across the column shows a significant difference ($p \leq 0.05$)

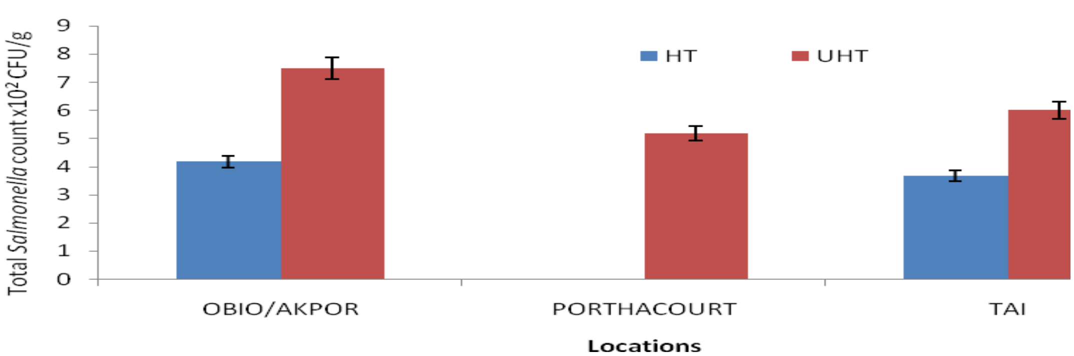

Figure 1: Total *Salmonella* Counts of the Healthy (HT) and Unhealthy Tomatoes (UHT) across the three LGAs

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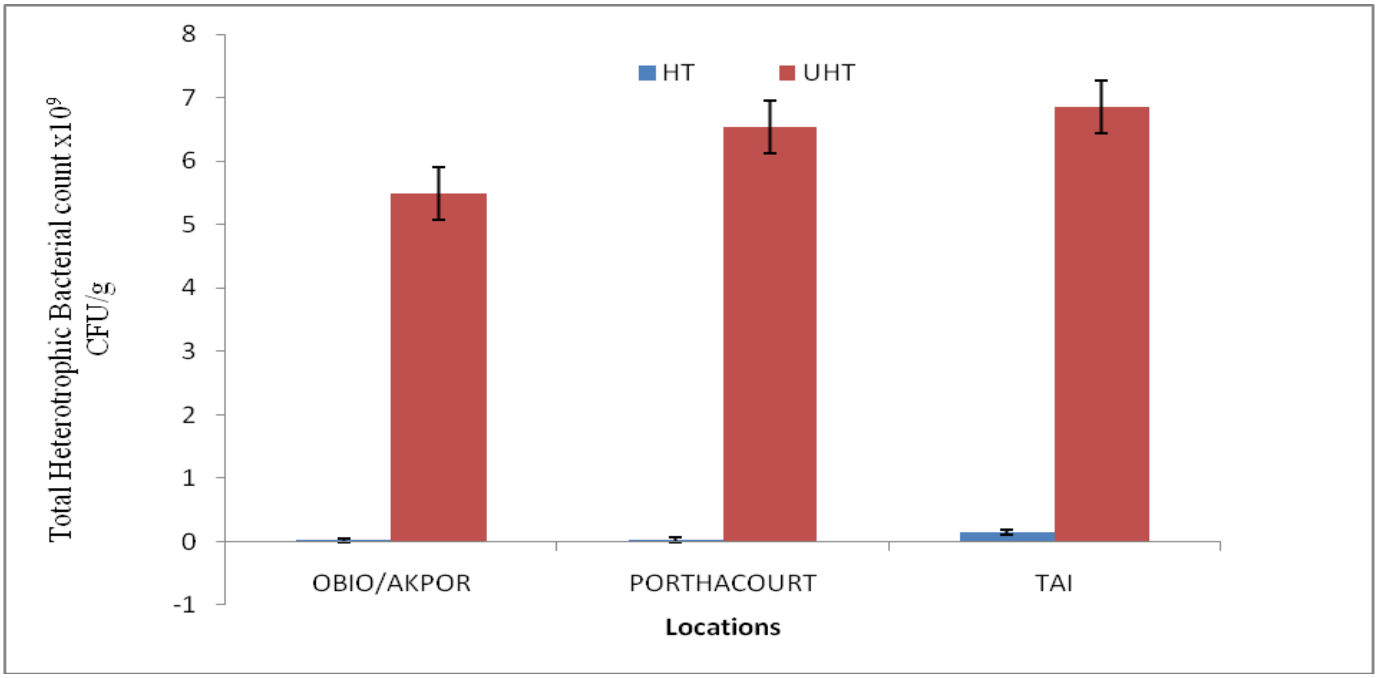

Figure 2: Total Heterotrophic Bacterial Counts of the Healthy (HT) and Unhealthy Tomatoes (UHT) the LGAs

The result of the prevalence of *Salmonella* spp from both healthy and unhealthy tomatoes across the three Local Government Areas is presented in Figure 3. *Salmonella enterica* (80%) was high in tomatoes from Port Harcourt when compared to Obio/Akpor (60%) and Tai (55%) respectively.

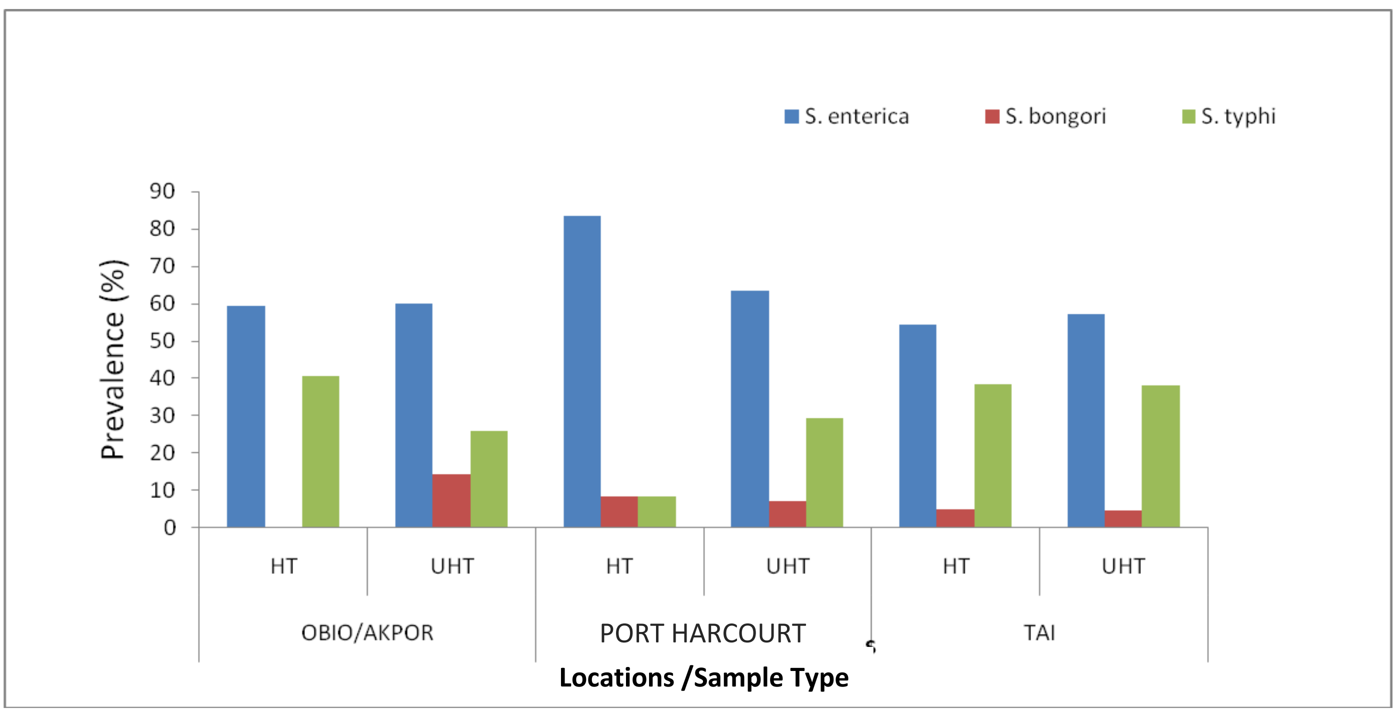

Figure 3: Prevalence of *Salmonella* from the Healthy (HT) and Unhealthy Tomatoes (UHT) in the LGAs

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The result of the susceptibility Pattern of *Salmonella* species isolated from tomato is shown in Table 8. *Salmonella enterica subsp. entericstrain* ON832663 and *Salmonella enterica subsp. enteric* AB681241 were susceptible to Ofloxacin and Levofloxacin (100%) respectively while *Salmonella bongori strain* MZ959600 and *Salmonella enteric strain* OP745459 showed a decreasing trend of resistance in the order Ampiclox and Ceftriazone- Sulbactam (92.9%) respectively.

The result of the Phenotype, Genotype, Accession number, Resistant Gene types (TEM and SHV) and MAR index of selected most resistant *Salmonella* are shown in Table 9. The isolates positive for TEM were *Salmonella enterica Subsp.enteric* AB681241 and *S. enteric strain* OP745459 whereas *S. bongori strain* MZ959600, *S. enterica Subsp.enteric* AB681241, *S. enterica strain* OP745459, *S. enterica Subsp.enterica strain* ON832663 and *S. enteric Subsp.enterica* AB594754 were positive for SHV gene. The other isolates were negative for TEM gene.

Table 3: Susceptibility Pattern of *Salmonella* species isolated from tomato

Antibiotics (Conc)	*Salmonella* spp (n=14)		
	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Ampiclox (10µg)	13(92.9)	0(0.00)	1(7.1)
Cefotaxime (25µg)	8(57.1)	4(28.6)	2(14.3)
Amoxicillin Clavulanate (30µg)	12(85.7)	0(0.00)	2(14.3)
Impenem/Cilastatin (10µg)	10(71.4)	1(7.1)	3(21.4)
Gentamicin (10µg)	12(85.7)	1(7.1)	1(7.1)
Ofloxacin (5µg)	0(0.00)	0(0.00)	14(100)
Cefexime (5µg)	9(64.3)	2(14.3)	3(21.4)
Nalidixic acid (30µg)	12(85.7)	1(7.1)	1(7.1)
Levofloxacin (5µg)	0(0.00)	0(0.00)	14(100)
CeftriazoneSulbactam (45µg)	13(92.9)	1(7.1)	0(0.00)
Cefuroxime (10µg)	10(71.4)	4(28.6)	0(0.00)
Nitrofurantoin (300µg)	10(71.4)	0(0.00)	4(28.6)

Table 9: Selected most Resistant Species of *Salmonella* with their Phenotype, Genotype, Accession number, Resistant Gene types (TEM & SHV) and MAR I

Codes	Phenotype	Genotype	Accession Number	Resistant Gene Types TEM	SHV	MAR Index
SE	*Salmonella* sp	*S. bongori*	MZ959600	_	+	0.5
SL	*Salmonella* sp	*S. enteric Subsp.enteric*	AB681241	+	+	0.6
SN	*Salmonella* sp	*S. enterica strain*	OP745459	+	+	0.5
SB	*Salmonella* sp	*S. enteric Subsp.enterica*	ON832663	_	+	0.7
SF	*Salmonella* sp	*S. enteric Subsp.enterica*	AB594754	_	+	0.7

Key: MAR: Multiple Antibiotic Resistance

Discussion

The bacteriological analysis of the tomato samples showed that the total heterotrophic bacterial (THB) counts had significant growth as they exceeded 10^5 CFU/g (Washington *et al*., 2006). More so, the findings further showed that the presence of bacteria on tomatoes enhanced the spoilage of those tomatoes which was similarly reported by Afreen *et al*. (2018).

This study showed that Tai LGA has the highest microbial load in both healthy and unhealthy tomatoes while Obio/Akpor had the highest number of total *Salmonella* in both healthy and unhealthy tomatoes. Factors contributing to this prevalence include environment of sales, person to person contact, improper washing of vending utensils (CDC, 2020). The high microbial load in these areas is of public health concern.

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The presence of bacteria in tomatoes bought from those markets may be because they were improperly handled during the sellers' attempt to arrange them for sales (Beuchat, 2006). The varying differences in contamination from the three Local Government Areas could also be as a result of difference in the sources of farm products or wholesale points where the market sellers bought their tomatoes from (Buck *et al*., 2003).

In this study, *Salmonella enterica* (80%) was the prevalent bacteria isolated from both healthy and unhealthy tomatoes. This is in contrast to Ugwu *et al.*'s (Ugwu *et al*., 2014) study in Nigeria which reported isolation rate of 8.9% in only unhealthy tomato and Wogu and Ofuase (2014) study from Benin City, Nigeria. The prevalence for *Salmonella enteric* (80%) was found to be higher than the 21.4% reported by Adebayo-Tayo *et al*. (2012), in Uyo Metropolis, Nigeria. Their presence in tomatoes may be due to handling practices by the vendors or from the soil in which tomatoes were planted or as a result of irrigation with contaminated waste water (Hintz *et al*., 2010). The high incidence of this bacterium is an indication of human contact, since improper handling of tomatoes during market days may have introduced this bacterium into the tomatoes (Etebu *et al*., 2013).

Furthermore, most of the isolates such as *Salmonella enterica subsp. enterica strain* ON832663 and *Salmonella enterica subsp. enteric* AB681241 were susceptible to Ofloxacin and Levofloxacin (100%) respectively.

However, high resistance was observed by *Salmonella bongori strain* MZ959600 and *Salmonella enteric strain* OP745459 to Ampiclox and Ceftriazone-Sulbactam (92.9%) respectively. The varying antibiotic prevalence has been previously reported by Wogu and Ofuase (2014) in a previous study on tomatoes in Benin City, Nigeria. The difference in resistance may be associated with varying functional groups of antibiotics and bacterial species. The presence of bacteria with antibiotic resistance associated with tomatoes sampled in this study highlights the potential risk of tomatoes to consumers.

The accession number and resistant genes of *Salmonella* isolates are presented in the Table 4. The isolates positive for TEM are *Salmonella enterica Subsp.enteric* AB681241 and *Salmonella enteric strain* OP745459 whereas *S. bongori strain*

MZ959600, *S. enterica Subsp.enteric* AB681241, *S. enterica strain* OP745459, *S. enterica Subsp.enterica strain* ON832663 and *S. enteric Subsp.enterica* AB594754 were positive for SHV gene. The remaining isolates were negative for TEM gene.

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