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Prevalence of *Salmonella* species contamination rate in some selected edible fruits in Osogbo, Osun state, Southern west, Nigeria

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ABSTRACT

The contribution of essential components of fresh fruits in human diet due to its medicinal and nutritional benefits associated with its consumption cannot be over-emphasized. This study was therefore designed to investigate the prevalence of *Salmonella* species contamination rate in some selected edible fruits in Osogbo, Osun state. A cross sectional study was conducted on two hundred and fifty (250) samples of three different conventional fruits namely Orange, Pawpaw and Watermelon. The dible fruits were analyzed for the presence, prevalence, susceptibility (sensitivity and resistant) patterns of *Salmonella* species and other bacteria to various antibiotics. The highest percentage of isolated *Salmonella* species was found in Watermelon (5.8%) followed by Orange (1.3%) and Pawpaw (1.2%). The most prevalent bacteria in all the fruits sampled were *Staphylococcus albus*. The multiple antibiotics resistance (MAR) and susceptibility pattern showed that Ofloxacin (100%) and Ciprofloxacin (100%) had the highest susceptible rate of *Salmonella* species isolated from the edible fruits. Other bacterium isolated includes *Staphylococcus albus*, *Escherichia coli*, *Shigella* species, *Staphylococcus aureus* and *Proteus* species. This investigation shows that ready to eat dible fruits must be washed thoroughly with clean water before eating to avoid been contaminated with microorganisms.

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1.0 Introduction

Freshly edible fruits are notably known for been an integral requirement for well-being in balance diet around the globe and provide essential nutrients such as vitamins, oil, minerals; fibers, low calories of fats in right proportion and many health benefits to human (Laventesi et al., 2012; Walsh et al., 2014; Reddy et al., 2016 and Gundappa and Gaddad, 2016). Adequate consumption of fruits is highly recommended so as to reduce the risk of diseases (Denis et al., 2016). Eni et al., (2010) observed that fruits and vegetables have ability to prevent scurvy, blindness and other infection.

Many compounds has been established to be present in fruits and these are antioxidants, folic acid, potassium, magnesium and non-nutritive bioactive constituents (phytoestrogens and other photochemical) (WHO, 2004). Consumption of fresh fruits was reported to improve good health despite their ability to harbor a good number of microbial contaminants such as Gram negative and Gram positive bacteria and also the fungi which includes the yeast and moulds (Eni et al., 2010). The major source of contamination is from the use of organic fertilizer such as manure, municipal sludge and faecal contaminated water while pathogen from human, animal and the environment are minor source of fruit contamination (Hanning et al., 2009).

Salmonella species are one of the different species of microorganisms associated with food poisoning (Ryan and Ray, 2004) and remain source of enteric fever. Among the species of Salmonella, *Salmonella enteritidis* and *Salmonella typhimurium* are the most frequently reported non-typhoidal serotypes in many countries causing food borne outbreaks due to association with a diverse range of food vehicles and most *Salmonella* infections are self-limiting, in a small proportion of cases (Fatica and Schneider, 2011). Enteric fever cause by Salmonella species is becoming re-occurrence in our environment and the source of the infection is necessary to be established. Hence this study is carried out to investigate the prevalence of *Salmonella* species contaminations in some selected edible fruits in Osogbo, Osun State, Nigeria.

2.0 Materials and methods

2.1 Survey and sample design

Edible fruits were collected between October, 2019 and March, 2020. For each of the three fresh-cut edible fruits; Pawpaw (85), Watermelon (85) and freshly peeled Orange (80) randomly selected from fruit vendors and hawkers from a wide range of markets located within Osogbo (Igbonna market, Kobongbogboe market, Oluode market and Orisunmbare market (Table 3.1). Edible fruits were collected from various fruit vendors ranging from producers to retailers.

The edible fruits were immediately moved to the laboratory after collection using an ice pack boxes. The samples were analyzed within 24 hours after collection. Spoiled fruits were exempted from the investigation. Each edible fruit sample was labeled with unique number and collection date. Large sample of edible fruits was used that will represent the targeted fresh-cut edible fruits available in Osogbo, Osun State during the time of the investigation.

2.2 Study area

This study was carried out in Osogbo, headquarters of Osogbo Local Government and the state capital city of Osun State. From north east of Ibadan, Osogbo is eighty-eight (88) kilometers by road, from south of Ilorin, hundred (100) kilometers by road and one hundred and fifteen (115) kilometers north-west of Akure (National Population Census, 2006).

2.3 Sample analysis

For the analysis, one (1) gram of each pre-cut fruit were transferred aseptically into a 10ml of sterile buffered peptone water in a sterile conical flask and homogenized by electric shaker. This was done to ensure the release and isolation of *Salmonella* spp. and other bacteria.

2.4 Isolation and identification of *Salmonella* spp. in suspension of pre-cut edible fruits

Suspensions of homogenized pawpaw, watermelon and orange in distilled water and in Selenite F broth culture were mixed thoroughly, streaked onto Xylose Lysine Deoxycholate agar plates (Oxoid) and incubated at 37°C for 24 hours. The growth on Xylose Lysine Deoxycholate plates were observed for black dot with red background and they were picked and subjected to Gram stain, motility, catalase, indole, urease, citrate, coagulase and nitrate test. For isolation of other bacteria, the remaining homogenized sample were sub-cultured onto MacConkey agar and blood agar and incubated at 37°C overnight.

2.5 Statistical analysis

Experiment was laid out in a 2x4 completely randomized design. All the data were subjected to Analysis of variance (ANOVA) using Statistical analysis system (SAS) user guide version 8.1, Statistical analysis system institute Inc., Cary. Means were separated using the least significant difference (LSD) at $P<0.05$.

3.0 Results and discussion

From Table 3.1 below, the result showed that of the pre-cut edible fruits sampled, watermelon 7 (5.8%) had the highest percentage of isolated *Salmonella* species while orange 1(1.3%) and pawpaw 1(1.2%) had the same percentage of isolated *Salmonella* species respectively. The highest percentage of isolated *Salmonella* species recorded by watermelon sampled could be as a result of the growth nature of watermelon having its fruits very close to the ground, hence can easily get contaminated with bacteria from the soil debris. This corroborates a previous study conducted by Nwachukwu et al., (2008) that reported that soil serves as source of contamination for fruits through the use of manure, watering of fruit with contaminated water and dropping from birds and animal.

Table 3.1: Occurrence of Salmonella species contamination in sampled edible fruits

S/N	Sampled fruits	No	No of isolated *Salmonella* Species	Percentage of Isolated *Salmonella* Species
1	Orange	80	1	1.3%
2	Pawpaw	85	1	1.2%
3	Watermelon	85	5	5.8%
	Total	**250**	**7**	**8.3%**

Table 3.2 shows the different types of Bacteria species isolated from the sampled pre-cut edible fruits. In this study, out of entire samples examined, sixty-five (65) samples were discovered to harbor at least one type of bacteria. Orange fruits had the highest occurrence rate of *Staphylococcus albus* 7(38.8%). This is followed by *Eschericia coli* 5(27.8%); In Pawpaw, the isolated bacteria with highest occurrence and contamination rate were *Staphylococcus albus* 8(34.8%); *Eschericia coli* 5(21.7%) and the least was *Proteus* species 1(4.4%). Watermelon equally recorded the highest occurrence rate of *Staphylococcus albus* 8(33.3%), followed by *Eschericia coli* 5(20.8%) and *Salmonella* species 5(20.8%) while *Staphylococcus aureus* 2(8.3%) had the lowest occurrence rate.

Furthermore, in all the pre-cut edible fruit sampled, *Staphylococcus albus* had the highest occurrence and contamination rate while *Proteus* species had the least occurrence in all the sampled fruits. This is as a result of the fact that *Staphylococcus albus* is a free bacterium of the skin and thus can be transferred easily from the contaminated hand of a fruit handler or vendor to the fruits (produce) with questionable personal hygiene condition.

Table 3.2: Different types of Bacteria isolated from edible fruits sampled

Detected Organisms	Prevalence rate Orange	Pawpaw	Watermelon
Salmonella species	1(15.6%)	3(13.0%)	5(20.8%)
Shigella species	2(11.1%)	4(17.4%)	4(16.8%)
S. aureus	2(11.1%)	2(8.7%)	2(8.3%)
S. albus	7(38.8%)	8(34.8%)	8(33.3%)
E. coli	5(27.8%)	5(21.7%)	5(20.8%)
Proteus species	1(5.6%)	1(4.4%)	0(0.0%)
Total	**18(100.0%)**	**23(100.0%)**	**24(100.0%)**

This results is in harmony with the works of (Fredlund et al., 1987; Beuchat, 1995; Little et al.,1998; Foley et al., 2006 and Jolaoso et al., 2010) whose study outcome reported that the presence of *Salmonella* species in pre-cut edible fruits could be due to fecal contamination of water; poor personal hygiene of handlers; use of contaminated packaging materials such as baskets, wheel barrows, processing equipment and trays to hawk / display these fruits may serve as possible source(s) of food contamination; improper storage, selling of left over fruits and easy accessibility of flies to the fruits during display.

In addition, the presence of *Eschericia coli* in the sampled pre-cut fruits indicates the possibilities of secondary contamination which is usually associated with fecal contamination. This finding agrees with Nwachukwu and Osuocha (2014) who reported that the presence of *Salmonella* species and *Eschericia coli* calls for concern as these organisms are frequently associated with poor sanitary practices and could be pointed to danger of possible food infections and can occur through unclean hands of the fruits vendors, packaging materials or from the contaminated soil.

Tables 3.3a, 3.3b and 3.3c present the antibiotic susceptibility pattern and multiple resistances to *Salmonella* species isolated from the sampled edible fruits. Tables 3.3a, 3.3b and 3.3c showed multiple antibiotics resistance (MAR) and susceptibility pattern of *Salmonella* species isolated from the sampled edible fruits to different doses of antibiotics respectively. Table 3.3b revealed that Ofloxacin 7(100.0%) and Ciprofloxacin 7(100.0%) had the highest susceptibility rate on *Salmonella* species isolated from all the sampled fruits. Followed by Pefloxacin 6(85.7%) and the least was Gentamicin 1(14.3%).

Table 3.3c showed that the highest percentages of resistant were recorded from Tetracycline 7(100.0%), Amoxycillin 7(100.0%), Nitrofurantoin 7(100.0%) and Augmentin 7(100.0%) and the least was with Ceftriazone 4(57.0%). The multiple antibiotics resistant (MAR) was calculated according to Krumperman (1985) formula:

$$MAR\ Index = \frac{\text{Total number of resistant antibiotics}}{\text{Total number of antibiotics applied}}$$

MAR index of less than 0.2 (< 0.2) indicate high risk source of contamination as a result f abuse use of antibiotics.

The results obtained as presented in Tables 3.3a, 3.3b and 3.3c showed that the rate of use of Ofloxacin and Ciprofloxacin is at lower rate while the rate of use of Tetracycline was at the highest rate. The high cost of broad-spectrum antibiotics like Ofloxacin and Ciprofloxacin may hinder its frequent usage by the fruits' vendors or hawkers. However, availability and accessibility of antibiotics like Tetracycline as a result of lower cost may account for the over usage of the treatment of any feverish condition like typhoid or enteric fever caused by *Salmonella* species by fruits handler, hence may cause increase in the resistant pattern of *Salmonella* species to Tetracycline.

Table 3.3a: Antibiotics susceptibility pattern and multiples antibiotics resistant (MAR) to *Salmonella* species' isolated from various types of edible fruits

Fruits	No	AGM	CFX	NOR	GTI	CMX	OFX	AMC	CPC	TTC	PFC	MAR
Orange	1	b	b	b	b	b	a	b	a	b	a	0.7
Pawpaw	1	b	a	b	b	b	a	b	a	b	a	0.6
Watermelon	5	b(5)	a(2) b (3)	b(5)	a(1) b(4)	a(2) b(3)	a(5)	b(5)	a(5)	b(5)	a(4) b(1)	0.5

Key; a = sensitive**,** b = resistant

Table 3.3b: Sensitivity pattern of *Salmonella* species to different antibiotics

Organism	Number	CFZ	GTI	OFX	CPC	PFC	CMX
Salmonella species	7	3	1	7	7	6	2
Percentage (%)	100%	42.8%	14.3%	100%	100%	85.7%	28.5%

Table 3.3c: Resistant pattern of *Salmonella* species to different antibiotics

Organism	Number	AGM	CFZ	NOR	GTI	CMX	AMC	TTC
Salmonella spp.	7	7	4	7	6	5	7	7
Percentage (%)	100%	100%	57%	100%	85.7%	71%	100%	100%

Key; AGM: Augmentin, **CFZ:** Ceftriazone**, NOR:** Nitrofurantoin**, GTI:** Gentamicin**, CMX:** Cotrimoxazole**, OFX:** Ofloxacin**, AMC:** Amoxycillin**, CPC:** Ciprofloxacin**, TTC:** Tetracycline**, PFC:** Pefloxacin**.**

Table 3.3d: *Salmonella* reaction to different identification test

	1	2	3	4	5	6	7	8	9	10	11	12
Salmonella	+	+	+	+	+	+	-	-	-	+	-	+
Salmonella ATCC 19114	+	acid	+	+	+	+	-	+	-	-	-	+

Key; 1 = catalase, 2 = glucose, 3 = maltose, 4 = motility, 5 = indole, 6 = mannitol, 7 = Gram reaction, 8= Voges Proskauer, 9= Oxidase, 10= Methyl red, 11 = lactose, 12= Hydrogen sulfite,

Like many tropical countries, intestinal bacteria are widely distributed in Osogbo, Osun State, Nigeria because of the climatic condition and probably poor personal hygiene. Also, the market chain from several hands ranging from producer and final consumer might promote contamination of edible fruits with various microorganisms. The incongruity between this study and other studies might be attributed to time of collection, temperature, differences in the sample size and the method used.

Furthermore, watermelon was found to showed higher level of *Salmonella* species 20.8% contamination than pawpaw (13.0%) and oranges (15.6%). The different observe in contamination rate between the watermelon and pawpaw might be might be due to the fact that watermelon is closer to the soil (Nwachukwu et al., 2008). Watermelons have been associated with enteric infections and multiple salmonellosis outbreaks (Walsh et al., 2014). The contaminated pericarp of the watermelon *Salmonella* spp can be transfer into the edible portions during cutting even with clean knife,

Salmonella spp, *Staphylococcus albus*, *Staphylococcus aureus*, Enterobacteriaceae and parasites are reported as source of food borne diseases (Mensah *et al*., 1999) and can be transmitted from unlicensed vended fruits vendors or local hawkers with little or no knowledge of good personal hygiene ((Barro *et al*., 2007; Muinde and Kuria, 2005).

Staphylococcus albus was the most associated bacteria in all the fruits used with a prevalence rate of 38.8% in orange fruits, 34.8% in pawpaw and 33.3% in watermelon, this finding correlate with earlier studies although, the percentage of contamination rate was not established This prevalence might be associated with the bacteria's cosmopolitan distribution in nature. Also, *Escherichia coli* were the second most prevalent contaminant with 27.8% in orange, 21.7% in pawpaw and 20.8% in watermelon species. *Escherichia coli* may occur due to human sewage or contaminated water.

Microbial isolates included *Staphylococcus albus*, *Salmonella* species, *Esherichia coli*, *Shigella* species, *Staphylococcus aureus* and *Proteus* species. *Staphylococcus albus* and *Esherichia coli* had the highest occurrence of 38.8% in orange samples, followed by *Esherichia coli* with 27.8%, *Salmonella* species 15.6%. Percentage occurrence of *Staphylococcus albus* in pawpaw was 34.8%, *Esherichia coli* was 21.7%, *Salmonella* species 13.0%. In watermelon, percentage occurrence of *Staphylococcus albus* was 33.3%, *Esherichia coli* was 21.7% and *Salmonella* species was 20.8%.

3.4 Conclusion

From the obtained results, this finding lends credence to Nwachukwu et al., (2008) who reported that soil serves as source of contamination for fruits through the use of manure, watering of fruit with contaminated water and also dropping from birds and animal and also Nwachukwu and Osuocha (2014) also claim that the presence of *Salmonella* species and *Eschericia coli* are frequently associated with poor sanitary practices and can occur through unclean hands of the fruits vendors, packaging materials or from the contaminated soil.

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