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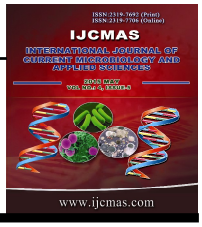

Original Research Article

Incidence of *Salmonella* and *Shigella* Species on some Selected Fruits and Vegetables Obtained from Open Area Markets in Calabar Metropolis

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A B S T R A C T

Consumption of fresh fruits and vegetables is in an increase demand because of their health benefits but the hazards associated with them are not taken notice of. Consumer demand is to use fresh fruits and vegetables which are bacteriologically safe but incidences have shown that eating these produce are not always safe. Survey of 390 samples of fresh fruits and vegetables among which were 100 each of cucumber (*Cucumis satavum*), garden egg (*Salinum melongena*) and carrot (*Daucus carota*) and 30 bundles each of pumpkin (*Telfairia occidentalis*), waterleaf T*alinum triangulare*) and afang (*Gnetum africanum*) bought from area markets for possible contamination with *Salmonella* and *Shigella* species were analysed. The high number of the pathogens on the surface of the fresh pumpkin, waterleaf, cucumber and garden egg is an indication of gross contamination of the fresh produce by pathogenic microorganisms. These range between 1.10 X 10^4 and 2.4 X 10^5 cfu/g. The higher incidence rate of these organisms also varies. The highest incidence of *Salmonella* spp (77%) and *Shigella* spp (57%) were found on the surface of waterleaf. Pumpkin followed with the incidence rate of 53% *Salmonella* spp and 33% *Shigella* spp. Carrot had only 5% incidence rate of *Salmonella* spp but 0% incidence rate of *Shigella* spp while afang had &% incidence rate of *Salmonella* spp but 0% *Shigella* spp. Salmonella spp was found to be the pre-dominant species in most of the samples. The loads and incidence rates of these pathogens on these produce show the contamination levels of these produce. The findings of this study show that most fruits and vegetables consumed in this part of the country are grossly contaminated with *Salmonella* spp and *Shigella* spp which are involved in food borne disease.

Keywords

Contamination, Food borne disease, Fruits, Pathogens, Vegetables, Incidence rate

Introduction

Fruits and vegetables make up part of the classes of foods that are responsible for the maintenance of physiological functioning of living cells as well as supply nourishment, growth, energy and tissue repair. Despite the benefits derived from fruits and vegetables,

they are major sources of food borne diseases (Robinson *et al.,* 2000). The number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables and unpasteurized fruit juices has increased in recent years (Buck *et al.,* 2003). More recently, salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe, mamey, apple juice and orange juice (Beuchat, 2002). There are also documented associations of shigellosis with lettuce, scallions and parsley (Martin *et al.,* 2003, Cook *et al.,* 1995). As can be seen above *Salmonella* and *Shigella* species have been associated with food borne diseases with significant morbidity and mortality rate in people of all ages. In the U.S., between 1995 and 1998, there were 9 outbreaks of food borne illnesses caused by *Salmonella* spp or *E. coli* 0157:H7 due to consumption of fresh vegetable sprouts (Martin *et al.,* 2003). All these reported cases are from Europe, U.S.A. and other developed countries result from eating raw fruits and vegetables. In this part of the world, there are no documented cases of illnesses resulting from eating fresh fruits and vegetables. This is not to say that people in this country do not get sick from eating these produce. It will therefore not be out of place to say that most common sicknesses in this country are caused by eating contaminated fruits and vegetables. Despite the efforts of the regulatory agencies to ascertain the safety of fruits and vegetables, the incidences have continued to rise in developed countries.

Afang (*Gnetum africanum*), waterleaf (*Talinum triangulare*), pumpkin (*Telfairia occidentalis*), carrot (*Daucus carota*), garden egg (*Solanum melongena*) and cucumber (*Cucumis sativus*) are common vegetables and fruits in this country. Some of them are eaten raw while others are mostly cooked half done. Again these produce are hawked along the highways and sold in open markets. Lennox and Efiuvwevwere (2012; 2013; 2014) in their work on cucumber and garden egg isolated *Salmonella* and *Shigella* species from these vegetables. These are food borne pathogens. There is therefore the possibility that they will carry and harbor pathogens that can cause food borne diseases. Handling of these produce in an unsanitary environments also make them vulnerable to gross contamination with food borne pathogens such as *Salmonella* and *Shigella* species. This study is therefore aimed at assessing the possible contamination of these produce with these pathogens.

Materials and Methods

Samples collection

A total of 390 fruit/vegetable samples were bought at random from the area local markets at different times for a period of 3 months (January, April and July). These comprised of 100 cucumbers, 100 garden eggs, 30 bundles of pumpkin, 30 bundles of afang, 30 bundles of waterleaf and 100 carrots which were placed in food grade bags and transported to the laboratory and analyzed immediately while others that could not be analyzed immediately were stored at 4^{o}C overnight before analysis.

Samples analysis

The total surface areas of the cucumber, garden egg and carrots were swabbed separately with moistened sterile swab sticks and the swab sticks were placed in 50ml of maximum recovery diluents (Williamson *et al.,* 2003). These were allowed to stand for 10 minutes.

For *Shigella* species isolation and identification: The method of Andrews and

Jacobson (2001) was used. Twenty five mills of the suspension was transferred into 225 ml of shigella broth containing 3.0 microgram per ml of novobiocin and incubated anaerobically for 20 hrs at 44^{o}C. After incubation, the suspension was shaken and streaked on MacConkey agar plates by spread plate method and incubated anaerobically at 42^{o}C for 24 hrs. The isolated colonies were characterized biochemically. Twenty grams each of the pumpkin, afang and waterleaf samples were separately rinsed in 130ml of maximum recovery diluents. These were shaken properly to dislodge any microorganisms that must have attached to the vegetables. These were allowed to stand for 5min. The method of Harrigan and McCance (1976) was used as stated above in the isolation and identification of *Shigella* species on these vegetables.

Salmonella isolation and identification

The method of Andrews *et al.* (2014) was used. Twenty five grams of each vegetable was weighed aseptically and placed into sterile wide mouth Erlenmeyer flasks containing 225 mL lactose broth. The contents were manually mixed by vigorously swirling the flasks for about 25 times clockwise and 25 times counterclockwise. The flasks were allowed to stand at room temperature for 60 ± 5.0 minutes. The pH were measured and adjusted to 6.8 ± 0.2 with 1N NaOH or 1N HCl, if necessary. These were incubated at 35º ± 2.0º C for 24 ± 2.0 hours. After the incubation, the flasks were shaken gently and 0.5 ml from each mixture was transferred to 10 ml Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml tetrathionate (TT) broth. The RV medium was incubated at 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically controlled, water bath) and the TT broth was incubated at 24 ± 2 h at 35 ± 2.0°C. These were mixed (vortexed,) and 10 µl from each incubated TT broth and RV medium were streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. After incubation, colonies typical of salmonella were picked, stocked and biochemically characterised. Fifty grams each of the cucumber, garden egg and carrot samples were aseptically obtained and placed separately in 450ml peptone/saline diluents and blended in a stomacher 400 (Techmar Co., Cincinnati, OH). Further 10-fold dilutions were prepared up to 10^{-4}. These were allowed to stand for 5 min. before culturing in duplicates. Also, 50g each of the fluted pumpkin, afang and waterleaf were separately blended in a stomacher 400 (Techmar Co., Cincinnati, Ohio). Each blend was placed in 450ml peptone/saline diluents and mixed properly. Further 10-fold dilutions were made up to 10^{-5}. Using spread plate method, 0.5ml aliquot of each suspension from 10^{-4} and 10^{-5} of each produce was pipette and placed on salmonella-shigella agar plates in triplicates using standard method of Mosupye and von Holy (1999). A curved glass rod was used to spread the contents in the plates. The glass rod was flamed after each use. The plates were incubated at 37oC for 18 hrs. At the end of incubation, the colony forming units from the plates having between 30 to 300 colonies been counted, averaged and total microbial loads of the produce were determined.

Results and Discussion

The mean aerobic counts per gram of the produce are shown in table 1 while the percent incidence rates of the organisms on the produce are shown in table 2.

The quality of fresh fruits and vegetables in this country is of great concern. It is very clear that the most important problem facing

the fresh produce industry is one of food safety. This study has shown that the produce studied was grossly contaminated with foodborne pathogens, *Salmonella* and *Shigella* species. This is in line with the works of Lennox and Efiuvwevwere, (2012, 2013 and 2014). The incidence rates of the two pathogens are highest in pumpkin and waterleaf. Also, these two vegetables showed very high microbial loads higher than the approved minimal levels. Some of these produce are consumed raw while others are cooked half-done before consumption. Changes in food consumption have brought to light unrecognized microbial food borne hazards. Fresh fruits and vegetables consumption has increased nearly 50% from 1970 to 1994 worldwide. In developing countries, where diarrhoeal diseases are particularly prevalent, determining the proportion due to food borne diseases can be difficult.

Clinical laboratory and public health infrastructure to perform such assessments is not always present. This makes it difficult to determine the food products that are responsible for most of the diarrhoeal diseases. Estimates show that each year two million people die from diarrhoeal diseases, mostly attributed to contaminated food and drinking water. Fresh produce is susceptible to contamination during growth especially near sanitary facilities, harvest, and distribution (Nguyen the and Carlin, 2000).

Table.1 Mean total *Salmonella* and *Shigella* species counts for each produce

Produce	Average cfu/g
Cucumber	$1.41 \times 10^4 \pm 0.03$
Garden egg	$1.2 \times 10^3 \pm 0.04$
Carrot	$2.4 \times 10 \pm 0.01$
Fluted pumpkin	$2.5 \times 10^5 \pm 0.03$
Waterleaf	$2.4 \times 10^5 \pm 0.02$
Afang	$1.4 \times 10 \pm 0.01$

Mean of 3 determinations

Table.2 Percent incidence rates of *Salmonella* and *Shigella* species

Produce	Organism Total No. detected Total samples Percent occurrence
Cucumber	*Salmonella* spp 43 100 43
	Shigella spp 21 100 21
Garden egg	*Salmonella* spp 34 100 34
	Shigella spp 14 100 14
Carrot	*Salmonella* spp 5 100 5
	Shigella spp ND 100 0
Pumpkin	*Salmonella* spp 16 30 bundles 53
	Shigella spp 10 30 ,, 33
Waterleaf	*Salmonella* spp 23 30 ,, 77
	Shigella spp 17 30 ,, 53
Afang	*Salmonella* spp 2 30 ,, 7
	Shigella spp ND 30 ,, 0

Mean of 3 determinations
Key: ND – not detected

Again, in the market places where they are sold, they are usually kept on the ground and tables where flies uncontrollable perch on (Beuchat, 1996; Hedberg and Osterholm, 1993; Sapers *et al.*, 2005). This calls for carefulness during growth, harvest and distribution. It is therefore necessary to wash and rinse these produce which may reduce the number of pathogens on the surfaces (Buck *et al.*, 2003).

Beuchat (1998) also stated that the effect of washing in reducing the number of bacteria present is small with reductions of 0.1 $1/\log_{10}$. The percent occurrence of the pathogens on the vegetables is an indication of the susceptibility of these produce to contamination by *Salmonella* and *Shigella* species and other food borne pathogens. The increase in the consumption of these produce has also increased food borne diseases caused by *Salmonella* and *Shigella* species. Pathogens on the surface of produce (e.g., melons) can contaminate the inner surface during cutting and multiply if the fruit is held at room temperature (Reis *et al.*, 1990).

Among the studied vegetables, waterleaf, pumpkin and cucumber are usually grown on soils contaminated with animal feces as manure or contaminated sewage. Over two-thirds of cases of shigellosis in the United States are caused by eating vegetables from a field contaminated with sewage. The studied vegetables are consumed widely in this part of the world and in an increasing manner. The problem here is lack of surveillance for food borne disease outbreaks which have made it difficult to document food borne diseases and outbreaks. With the occurrence of 5–77% of Salmonella species on the studied produce, it is likely that consumption of these vegetables will in most cases result in food borne diseases. This is in agreement with the studies carried out in the United States from 1990 to 1997, which reported that increased consumption of fresh produce may have contributed to a series of food borne outbreaks associated with foods such as sliced cantaloupe Reis *et al.* (1990), green onions Cook *et al.* (1995), unpasteurized cider Besser *et al.* (1993), fresh squeezed orange juice Cook *et al.* (1996), lettuce Acker *et al.* (1996), raspberries Herwaldt and Ackers (1997), alfalfa sprouts Mahon *et al.* (1997), sliced tomatoes Wood *et al.* (1991), Reller *et al.* (2006). *Shigella* spp occurred in low percentages but it is important to know that infectious dose of 100 cells of *Shigella* species or less consumed can cause food borne disease. Its occurrence ranged between 0 and 53%. The loads and incidence of these pathogens on the produce show the safety levels of these produce and cause for concern, particularly given the serious morbidity and mortality that can be associated with these pathogens. The surface of vegetables and fruits may be contaminated by human or animal feces as can be seen from this study. It is therefore necessary to take adequate safety precaution before eating any of these vegetables.

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