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The Microbial Contamination of Ready-To-Eat Vended Fruits in Abakpa Main Market, Abakaliki, Ebonyi State, Nigeria

[1]Orji Jerry Okechukwu, [1]Orinya Chinedu I., [1]Okonkwo Eucharia O., [2]Uzoh Chukwuma V., [1]Ekuma Uchechukwu O., [3]Ibiam Gideon Ama And [4]Onuh Euslar N.

[1]*Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria.*
[2]*Department of Biology, Microbiology and Biotechnology Faculty of Science, Federal University Ndufu-Alike Ikwo, Nigeria.*
[3]*Department of Medical Laboratory Science, Ebonyi State University Abakaliki, Nigeria.*
[4]*Ebonyi State School of Health Mgbo, Ohaukwu Local Government Area, Ebonyi State, Nigeria.*

Abstract: *The microbial contamination of ready-to-eat vended fruits in Abakpa Main market, Abakaliki was examined using standard microbiological methods. A total of thirty (17) samples of vended fruits were screened for total bacterial and fungal count. From examination five (5) bacterial species were isolated namely: Escherichia coli, Staphylococcus aureus, Salmonella sp, Shigella sp and Pseudomonas sp while one (1) fungal species, Mucor sp, was isolated from the vended fruit samples. The total aerobic plate count ranged from 3.5×10^5-1.03×10^6 CFU ml^{-1} with tiger nuts having the highest count and cucumber having the lowest count. The total fungal count ranges from 1.1×10^5- 1.42×10^6 CFU ml^{-1} with carrot having the highest count and pineapple (sliced) having the lowest count apart from tiger nuts that had no significant growth. The isolated organisms from the vended fruits showed that contamination occurred due to poor hygiene and environmental factors like contaminated air. Therefore adequate tutorials on sanitary practices on both individuals and environment should be encouraged by concerned government officials to reduce the level of contamination in vended fruits.*

I. Introduction

Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are also vital for health and well- being. Well balanced diets, rich in fruits have been reported to help to prevent Vitamin C and Vitamin A deficiencies and to reduce the risk of several diseases (Kalia and Gupta, 2006). Fruits are widely exposed to microbial contamination through contact with soil, dust and water and also by mishandling during harvest or post harvest processing. They therefore harbour a diverse range of micro-organisms including pathogens (Kalia and Gupta, 2006).

Vended fruits are fruits that have been cut or sliced open and carried around by street vendors or hawkers at local markets or streets and such fruits are eaten immediately that is they are eaten without necessarily having to cut, peel or rinse them before consumption because they have already been prepared or packaged by the vendors (Kaplan and Campbell, 1982; Lund, 1992; De Roever, 1998). They are usually packaged in small polyethene bags for sale.

Over the last few years, there has been a significant increase in the consumption of vended fruits in Nigeria. This is because they are easily accessible, conveniently, and most importantly, they are cheaper than the whole fruits. Other reasons include modern lifestyle, industrialisation, economic downturn, materialism and lack of time to prepare proper meal (Nielsen, 2006). The increased consumption coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern. Most times, it is difficult for one to attest to the hygiene of the processors or the sanitary conditions during preparation. This is worsened by the fact that vended fruits are done without adequate storage conditions, thereby, exposing the fruits to flies, dust and other pathogens (Barro *et al.,* 2007). These vended fruits such as watermelon, pineapple, carrots, cucumber, tiger nuts (also known as *aki hausa*) are sold by unlicensed vendors or local hawkers who have little or no knowledge on food hygiene (Muinde and Kuria, 2005). This therefore increases the risk of food-borne diseases caused by a wide range of pathogens such as bacteria (*Salmonella sp, Staphylococcus aureus, Enterobacteriaceae*), fungi, viruses and parasites (Mensah *et al.,* 1999). And these pathogens could invade these fruits during washing, peeling, slicing, trimming, packaging, handling and marketing (Barro *et al.,* 2007; Khali *et al.,* 2007). The use of dirty utensils encourages rare visits of cockroaches, flies and rats (Bryan *et al.,* 1992).

Aim Of The Study

To assess the microbial contaminants of some vended fruits sold in Abakpa, Main Market, Abakaliki, Ebonyi State, Nigeria.

Objectives Of The Study

Determination of total aerobic bacteria plate count and total fungal count.
Isolation and identification of bacteria and fungi contaminants from the different ready-to-eat vended fruits.
Determination of the percentage frequency distribution of the microbial isolates on the ready-to-eat vended fruits.

Statement Of Problem

Fruits continue to remain a source of nutrients that are very essential to human health and these nutrients help to reduce the risk of some life threatening diseases like cancer and cardiovascular diseases. Fruits have played a good role in the human health and that is why today they are highly purchased in the markets and streets as well. Some of these fruits are sold in a packaged way that the buyer or consumer does not need to wash or cut the fruits before eating. And most of these vendors are in experienced based on personal hygiene that is to say that during processing, these ready-to-eat vended fruits are exposed to contaminated air, unclean utensils and unclean environment. This is why it is necessary to check for the microbial contamination of such fruits to have an insight on the risk people expose their health to these vended fruits when purchased.

Significance

This study will help give an idea on how contamination of these ready-to-eat vended fruits can pose a threat to human health based on food poisoning.
It will also create awareness to the role of concerned government health officials in the control of microbial contamination in these ready-to-eat vended fruits.

II. Materials And Methods

Study Area

This study was conducted in Applied Microbiology Laboratory Unit, Ebonyi State University, Abakaliki while the samples were collected from different fruit vendors in Abakpa Main Market, Abakaliki, Ebonyi State. Abakpa Main Market, Abakaliki, also known as "meat market" is the largest market in Ebonyi State with different people selling different items like foodstuffs, fruits, vegetables, wears and other exciting goods. A great number of traders there are involved in fruit selling. And most of them are sliced or processed because most of their customers may not be able to afford or have time to process the fruits properly.

Materials and Reagents

The materials and reagents used during the course of this research include: weighing balance, beakers, conical flasks, autoclave, petri-dishes, 70% ethanol, non-absorbent cotton wool, aluminium foil, test tubes, wire loops, incubators, microscope, blender, nutrient agar, potato dextrose agar, mannitol salt agar, salmonella-shigella agar, macConkey agar, peptone water and distilled water.

Collection of samples

A total of seventeen (17) vended fruit samples consisting of carrot, cucumber, tiger nuts, sliced watermelon and pineapple were collected. The sliced watermelon and carrot were collected from four different fruit vendors while the cucumber, sliced pineapple and tigernuts were collected from three different fruit vendors. They were all collected and put into different white polyethene bags to differentiate them based on the vendors they were bought from.

Analysis procedure

Media preparation: The different media which included nutrient agar, potato dextrose agar, mannitol salt agar, macConkey agar and salmonella-shigella agar; and peptone water were prepared according to the manufacturer's instruction.

Isolation of micro-organisms from the vended fruit samples: About 10g of each of the fruit samples were weighed and homogenised in 90ml of sterile distilled water using an electric blender. Then, ten-fold dilutions of the homogenates were made with sterilized peptone water; after that 1ml of the 10^{-4} dilutions of thehomogenates were dispensed into the petri-dishes that were labelled based on the agar used by pour plate method and allowed to gel. After gelling, the petri-dishes that contained mannitol salt agar, nutrient agar, macConkey agar and salmonella-shigella agar were incubated at 37°C for 24hours while the petri-dishes that contained potato dextrose agar were incubated at 25°C for 3days.

The nutrient agar, macConkey agar, mannitol salt agar and salmonella-shigella agar were used to check for total bacterial count, total coliform count, presence of *Staphylococcus aureus*, *Salmonella* and *Shigella spp* respectively.

At the end of the incubation period, the plates were brought out of the incubators and the colonies were counted using a colony counter device and each count was expressed in colony forming unit per ml (CFU ml^{-}).

Isolation of the cultured micro-organisms

The distinct colonies on nutrient agar and potato dextrose agar were carefully examined using microscope for their morphological characteristics like colour. Then these colonies were subcultured on nutrient agar using streaking method and were incubated at 37°C for 24hours.

Identification of Isolates

Gram staining and other biochemical tests were carried out based on the method of Cheesbrough (2006). The biochemical tests performed here included catalase test, oxidase test, indole test and coagulase test.

Biochemical tests

Catalase test: The discrete colonies of each of the isolates were collected with a wooden stick and emulsified in a drop of hydrogen perioxide (H_2O_2). Bubbles of gas indicated a positive result according to Cheesbrough (2006).

Indole test: Here a little portion of each of the isolates was inoculated into 5ml of sterilised prepared peptone water which was contained in different test tubes using a wire loop. And then, the testtubes containing the organisms were left to incubate at 37°C for 48hours. After incubation period, 3-4drops of indole reagent known as Kovac's reagent was added and shook gently. A positive result gave a red surface layer after 10minutes while a negative result gave no red surface layer after 10minutes according toCheesbrough (2006).

Oxidase test: A piece of filter paper was placed in a clean petri dish and 2-3drops of freshly prepared oxidase reagent was added. With the aid of a wooden stick, discrete colonies of the isolates were collected separately and smeared on the filter paper. A positive result gave a purple-blue colouration after 10seconds while a negative result gave no such colour after 10seconds according to Cheesbrough (2006).

Coagulase test:A drop of distilled water was placed on each end of a slide and a colony of the test organism was emulsified in each of the drops to form a thick suspension. Then a loopful of plasma was added to one of the suspensions and swirled gently. A positive result showed clumping after 10secconds while a negative result showed no clumping after 10secondsaccording to Cheesbrough (2006).

Gram staining:A thin smear of the isolates were made on different slides with the aid of a wire loop and left to dry and after they were heat fixed and allowed to cool. Then the different smears were covered with crystal violet stain for 30-60seconds and rapidly washed off with clean water. Then the smears were covered with Lugol's iodine for 30-60seconds and rapidly washed off with clean water. The smears were decolourised rapidly with alcohol and washed out immediately with clean water. Then the smears were covered with safaranine for 30-60seconds and washed immediately with clean water. The stained smears were then allowed to air-dry. After drying, a few drops of oil immersion were dropped on the stained smears and viewed with the aid of a microscope (×100 oil objective lens) to check for the microscopic properties of the organisms like the Gram reaction, morphology (Cheesbrough, 2006).

For the fungal isolate, a drop of lactophenol cotton blue stain was dropped in the centre of a clean slide. And then a fragment of the fungus was collected with the aid of a wireloop and placed in the drop of the stain and teased gently and covered with a coverslip. The coverslip was not pushed down or tapped to avoid the dislodging of the conidia from the conidiophores. Then the stained isolate was viewed under the microscope with ×10 and ×40 objective lens for its morphological characteristics (Cheesbrough, 2006).

III. Results

The results of the microbial contamination of the processed vended fruit samples collected from different fruit vendors in Abakpa Main market, Abakaliki are presented in the following tables.

Table 1 shows the result of the average microbial load of the vended fruit samples in Colony forming unit per ml (CFU ml^{-1}). It reveals that tiger nuts has the highest average total aerobic plate count of 1.03×10^6, followed by watermelon (sliced), 1.0×10^6, while cucumber has the lowest, 3.5×10^5. Moreover, in the total fungal count carrot has the highest of 1.42×10^6, followed by watermelon, 1.26×10^6. No significant fungal growth was observed in tiger nuts.

Table 2 shows the result of the morphological and biochemical characteristics of the microbial isolates from the ready-to-eat vended fruit samples. It reveals that a total of six (6) micro-organisms were isolated. Out of these isolates, five were bacterial isolates namely: *Salmonella sp*, *Pseudomonas sp*, *Escherichia coli*, *Shigella sp* and *Staphylococcus aureus*; and one fungal isolate, *Mucor sp*. All the bacterial isolates are rod-shaped and Gram negative except for *Staphylococcus aureus* that is cocci in shape and Gram positive. Moreover, all bacterial isolates are catalase positive while four bacterial isolates are coagulase positive except for *Pseudomonas sp* which is coagulase negative.

Figure 1 shows the percentage frequency of occurrence of the microbial isolates in the ready-to-eat vended fruit samples. It shows that *Escherichia coli* has the highest occurrence, 5(83.3%), followed by *Salmonella sp*, *Staphylococcus aureus* and *Mucor sp*with 4(66.7%) respectively. *Pseudomonas sp*and *Shigella sp* has the least occurrence of 2(33.3%).

Table 1: Average microbial load of ready-to-eat vended fruit samples (CFU ml^{-1})

Vended Fruit Samples	Vendors	Total Aerobic Plate Count (TAPC)	Average TAPC	Total Fungal Count (TFC)	Average TFC
Carrot	A	9.6×10^5	7.97×10^5	1.44×10^6	1.42×10^6
	B	6.7×10^5		TNTC	
	C	TNTC		8.3×10^5	
	D	7.6×10^5		1.99×10^6	
Watermelon (sliced)	E	TNTC	1.0×10^6	1.77×10^6	1.26×10^5
	F	1.6×10^6		6.4×10^5	
	G	9.7×10^5		2.02×10^6	
	H	4.4×10^5		6.1×10^5	
Pineapple (sliced)	I	4.2×10^5	5.6×10^5	TNTC	1.1×10^5
	J	9.0×10^4		1.1×10^5	
	K	1.17×10^6		TNTC	
Cucumber	L	3.2×10^5	3.5×10^5	4.5×10^5	3.0×10^5
	M	5.4×10^5		NG	
	N	1.8×10^5		9×10^4	
Tiger nuts	O	1.04×10^6	1.03×10^6	NG	NG
	P	9.4×10^5		NG	
	Q	1.12×10^6		NG	

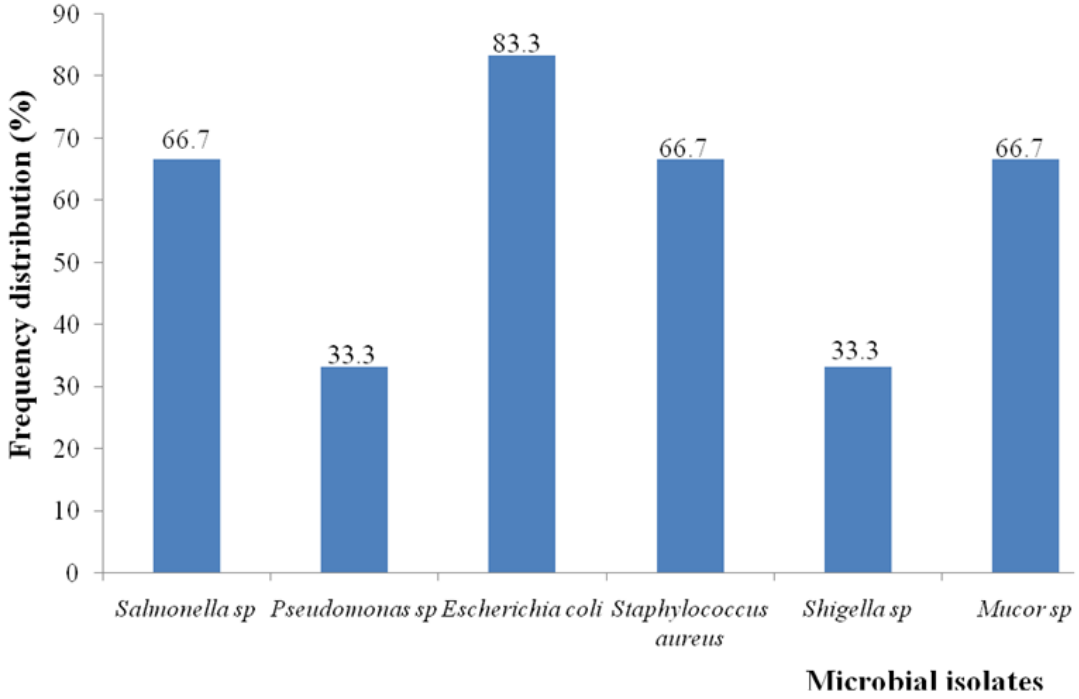
Keys: TNTC: To Numerous To Count
NG: No Growth

Table 2: Morphological and Biochemical characteristics of the microbial isolates from the ready-to-eat vended fruit samples.

S/N	Vended Fruit samples	Biochemical test				GRAM REACTION			Morphological characteristics	Probable organisms
		CA	CO	OX	IND	+ve/-ve	Shape	Arrangement		
1	Carrot	+	-	-	-	-	Rod	Single	Pale white with black edges	*Salmonella species*
		+	+	+	-	-	Rod	Single	Yellow-green	*Pseudomonas species*
		+	-	-	+	-	Rod	Single	Pink	*Escherichia coli*
		+	-	+	+	+	Cocci	Group	Yellow	*Staphylococcus aureus*
									Cottony dark-grey branched with round sporangiospores	*Mucor species*
2	Watermelon (Sliced)	+	-	-	+	-	Rod	Single	Pink	*Escherichia coli*
		+	-	-	-	-	Rod	Single	Pale white with black edges	*Salmonella species*
		+	-	+	+	+	Cocci	Single	Yellow	*Staphylococcus aureus*
		+	-	-	-	-	Rod	Single	Pale white	*Shigella species*
									Cottony dark-grey branched with round sporangiospores	*Mucor species*
3	Pineapple (Sliced)	+	-	-	+	-	Rod	Single	Pink	*Escherichia coli*
		+	-	-	-	-	Rod	Single	Pale white with black edges	*Salmonella species*
		+	-	+	+	+	Cocci	Group	Yellow	*Staphylococcus aureus*
		+	-	-	-	-	Rod	Single	Pale white	*Shigella species*
									Cottony dark-grey branched with round sporangiospores	*Mucor species*
4	Cucumber	+	-	-	+	-	Rod	Single	Pink	*Escherichia coli*
		+	-	+	+	+	Cocci	Group	Yellow	*Staphylococcus aureus*
									Cottony dark-grey branched with round sporangiospores	*Mucor species*
5	Tigernuts	+	-	-	+	-	Rod	Single	Pink	*Escherichia coli*
		+	+	+	-	-	Rod	Single	Yellow-green	*Pseudomonas sp*
		+	-	-	-	-	Rod	Single	Pale white with black edges	*Salmonella species*

Keys: CA= Catalase test, + = Positive, CO= Coagulase test, − = Negative, OX= Oxidase test, IND= Indole test

Figure 1: Frequency distribution (%) of the microbial isolates on the ready-to-eat vended fruit samples.



IV. Discussion

Bacteria and fungi are the common contaminants of our fruits and they could be easily transferred from the vendors to the processed fruits through mishandling. The consumption of ready-to-eat fruits directly from street vendors or hawkers potentially increase the risk of food-borne diseases caused by a wide variety of pathogens, because it is difficult to attest to the hygiene of these vendors or to the sanitary conditions at points of processing as well as the packaging materials. This could pose a threat to human health and this helps to throw light to the microbial contamination of ready-to-eat vended fruits that were collected from different fruit vendors in Abakpa Main market, Abakaliki.

These micro-organisms isolated were *Escherichia coli* (83.3%), *Salmonella sp* (66.7%), *Pseudomonas sp* (33.3%), *Staphylococcus aureus*(66.7%), *Shigella sp* (33.3%) *and Mucor sp* (66.7%). All the microbial isolates apart from *Shigella sp* was reported in the work of Odebisi-Omokanye *et al.,* (2015) in the microbial quality of pre-cut fruits sold in Ilorin, Kwara state; Jolaoso *et al*.,(2010) isolated *Staphylococcus aureus, Salmonella sp* and *Escherichia coli* from sliced pineapple and paw-paw. This is further supported by the work of Oranusi and Olurunfemi, (2011) that isolated *Staphylococcus aureus, Pseudomonas sp, Salmonella sp* and *Escherichia coli* from ready-to-eat fruits sold in Otta, Ogun state; Tambeker *et al*., (2009) also isolated *Staphylococcus aureus, Pseudomonas sp, Salmonella sp* and *Escherichia coli* from street vended fruits juices in Amravati, India. Moreover, the result of this study is in line with the report of Fowoyo, (2012) from air-contaminated vended foods sold in Lokoja, Kogi state.

Most of the isolates in this study may have been introduced into these fruits through faecally polluted water used in washing utensils like knives, trays and polyethene bags used for the packaging of the fruits after slicing or cutting and also exposure of these fruits to low temperatures which encourage the microbial growth of these pathogens (Daniyan and Ajibo, 2011). The presence of *Staphylococcus aureus, Pseudomonas sp, Salmonella sp* and *Escherichia coli* was in line with the work of Odebisi-Omokanye *et al.,* (2015) from pre-cut fruits sold in Ilorin. *Staphylococcus aureus, Salmonella sp, Shigella sp, Pseudomonas sp* and *Escherichia coli* are environmental isolates and they have been isolated from plants, human skin, animal and dairy products. Their presence in these ready-to-eat fruits may have been through unclean hands of the vendors, contact with sewage and contaminated water (De Roever, 1998). This implies that the fruit samples could serve as a vehicle in the transmission of these pathogens to the consumers of these contaminated fruits.

The presence of *Staphylococcus aureus* may have been introduced into the ready-to-eat fruits through body contact of vendors with the fruits because the organism is a normal flora of the nasal passage, hands and skins of healthy individuals (Nester *et al*., (2006). Odebisi-Omokanye*et al*., (2015) and Ganguli, (2006) reported *Staphylococcus aureus* to have the highest occurrence in fruits and foods respectively. It was recorded to be the second highest occurring isolate with the frequency of occurrence of 4(66.7%). Aboloma, (2008) and Wada-Kura *et al*., (2009) have also reported that the incidence of *Staphylococcus aureus* in food is an indication of

environmental and human contamination. This high incidence may have occurred due to the use of polyethene bags for the packaging of these fruits after slicing or cutting them (Little and Mitchell, 2004).

In this study, *Mucor sp, Salmonella sp* and *Staphylococcus aureus* had the same incidence of 4(66.7%). Oviasogie *et al*., (2015) reported such incidence of *Mucor sp*in the assessment of fungal pathogens associated with orange spoilage sold in Benin, Edo state while Oluwatoyin *et al*., (2015) reported such high incidence in *Salmonella sp* and *Staphylococcus aureus* in assessment of the microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, Ogun state. The presence of *Mucor sp* promotes the contamination and because they are ubiquitous they can be found on fresh vegetables, fruits and other substances that give nutrients. They are also able to withstand high concentration of sugar and they can survive in the absence of water or moisture. Such high occurrence may have occurred as a result of the exposure of these ready-to-eat fruits to dusty or muddy areas. Most of these fruit vendors stay near stagnant water of gutters which may serve as an entry for fruit contamination. Frank and Warribor, (2006) reported that the microbial load on leafy vegetables and fruits increase with time during storage. When these fruits are stored at inappropriate temperatures, they tend to attain temperatures that are suitable for the microbial growth of these pathogens to cause diseases when ingested (Bryan *et al*., 1992; Muinde and Kuria, 2005).

The results show that *Escherichia coli* had the highest frequency of occurrence of 5(83.3%) and it conforms to the report by Daniyan and Ajibo, (2011) and Daniel *et al*., (2014) in sliced fresh fruits sold in Minna and Bida metropolis respectively. *Escherichia coli* is regarded as primary indicator for microbiological quality of food and water and this shows that these fruits are not safe for human consumption. According to CDC, (2011), the main transmission of *Escherichia coli* was through faecally contaminated food or water. The high occurrence may have occurred in the contact of contaminated water with the fruits during washing of the fruits and also the inadequate washing of hands by the fruit vendors (Tambekar *et al*., 2007). Some of these fruit vendors get their water from unclean sources like dirty streams and also they could use very little quantity of water to wash or rinse all the fruits. The low occurrence of *Pseudomonas sp* and *Shigella sp* was also reported by Fowoyo, (2012) in the assessment of air contaminated vended foods sold in Lokoja, Kogi state.

These ready-to-eat fruits may get contaminated from knives used for cutting or slicing, improper human handling and processing, tables or trays used during peeling and cutting, rinsed water, washing buckets and packaging materials as these fruits are cut, washed, wrapped with transparent polyethene bags and sold to the consumers. The presence of these possible pathogens in the analysed fruit samples should be of great importance to the vendors, consumers and concerned arms of government.

V. Conclusion

In conclusion, the result from this study has shown that poor hygiene of the vendors and environmental factors could cause the microbial contamination of these processed vended fruits sold in Abakpa Main market, Abakaliki. From time to time, government health officials should give attention to the market especially these fruit vendors, at least to put on check how these vended fruits are processed which includes the type and source of water used, the condition of the utensils and most especially the personal hygiene of the fruit vendor to reduce help the rate of vended fruit contamination. Public awareness programs can also be used as a measure to educate these fruit vendors on personal and environmental hygiene to reduce contamination.

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