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A STUDY OF MICROBIAL ANALYSIS OF FRESH FRUITS AND VEGETABLES, IN SAGAMU MARKETS SOUTH-WEST, NIGERIA.

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ABSTRACTS

Raw and minimally processed fruits and vegetables are essential parts of diets of people around the world. However, these food items have consistently served as vehicles for human diseases worldwide. This study was aimed at determining the microbiological quality of fresh fruits and vegetables sold at Sagamu markets, Ogun state, Nigeria. Twenty different samples of fruits and vegetables were randomly purchased from ten various vendors in Shagamu market, Ogun State, Nigeria. Aerobic plate counts, total coliform counts, yeast, mold counts and antimicrobial susceptibility study using were determined using standard microbiological methods. In Fruits, the isolation rate of Bacillus specie was predominant (44.1%), followed by Klebsiella species (44.0%) while in vegetables, the prevalent rates of 38.3% and 14.4% were respectively recorded by Staphylococcus aureus and Pseudomonas species. Malassezia species and Aspergillus species were the only fungal isolates obtained from the fruits and vegetables. Aspergillus species were predominant in vegetables, with a frequency of 13.3% while both Malassezia and Aspergillus species were observed almost at the same frequently of 24.1% and 24.4% in fruits respectively. This study showed that fruits and vegetable were contaminated with potential food borne pathogens with varying degree of antibiotic resistance thus suggesting that they could act as a vehicle for the dissemination of antibiotic resistant organisms as well as serve as a reservoir of food borne pathogens of public health significance.

Keywords: Micro flora, fresh fruits and vegetables, antibiotics resistance,

INTRODUCTION

Fresh fruits and vegetables are a constant part of the daily diets of Nigerians, and are known for their high nutritional and health values, with fruits and vegetables being excellent source of essential nutrients (Senjuti *et al*., 2014). However, the contamination of fresh produce is a major public concern, as food borne diseases are increasingly becoming a global public health problem (Hannan *et al*., 2014), resulting in a tangible amount of morbidity and mortality annually worldwide (Hanson *et al*., 2012). Also the agricultural sector of Nigeria is a major contributor to its development (Fatoba *et al*., 2011) thus food loss is a major concern. Microbial contamination of fruits and vegetables regularly occurs in plantation fields, contact with soil, dust, contaminated irrigation water and use of raw sewage or manure fertilizers (Jung *et al*., 2014; Razzaq *et al*., 2014; Senjuti *et al*., 2014; Hannan *et al*., 2014). The process of transporting these food products from the farm to households also contributes to the contamination of these fruits and vegetables, thus posing a serious problem in food safety (Isa *et al*., 2014).

Various pathogens are associated with the contamination of fruits and vegetables, with different outbreaks of gastroenteritis related, associated with the consumption of contaminated vegetables have been recorded at various times (Jeddi *et al*., 2014). Pathogens implicated in contamination of fruits and vegetables include; *Escherichia coli, Salmonella* spp., *Listeria monocytogenes, Aeromonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Vibrio* spp. and *Pseudomonas* spp (Nwachukwu and Chukwu, 2013; Senjuti *et al*., 2014). In developing countries, food borne illness caused by contaminated fruits and vegetables are frequent but rarely properly documented and reported due to many reasons such as poor diagnostic facilities and lack of food borne disease investigation and surveillance. In Nigeria, Hazard Analytic Critical Control Point (HACCP) is not implemented thus, fruits and vegetables are grown with untreated human and animal wastes, transported and sold by local farmers or retail outlets for further processing by street vendors, by families at home or as part of meals eaten in restaurant and other eatery outlets (Eni *et al*., 2010), with this practice being pointed out to be one of the major contributing factors to high level of contamination of farm produce in Nigeria (Olayemi, 2007).

Studies of vegetables and fruits microbial contamination consumed by most people and their degree of resistance against a panel of antibiotics, will give an indication as to the resistance pattern of bacteria associated with raw vegetables and fruits in the study area, and can help identify potential sources of drug-resistant strains in Nigeria. Results obtained from this study can also be referred to in order to draw inference about the conditions of farm produce in Nigeria and possible links with spread to humans.

MATERIALS AND METHODS

Sample collection and processing

Sagamu is a popular town in Ogun State and one of the fast growing towns in South- west Nigeria. A total of Four hundred samples of assorted kinds of fruits and vegetables were purchased from different sellers at different fruit markets in Sagamu. 20 types of each fruit from 11 different handlers (220) and 20 types of each vegetable from 9 different handlers (180) were procured from these markets. To prevent post service contamination, the market sellers were instructed to dispense the samples into pre-sterilized metallic containers with lids. All samples were labelled and transported to the Medical Microbiology and Parasitology Laboratory of Olabisi Olabanjo University, Sagamu, for immediate processing. Fruits and vegetable samples were first swab in Nutrient Broth, and Selenite F broth overnight and then culture on, Blood Agar, MacConkey Agar, Manittol Salt Agar, and for Selenite F' Broth was culture on Salmonella Shigella Agar, for fungi isolation also on Potato Dextrose Agar for myco-flora isolation. Microbial identification was carried out in line with the Standard Methods for Microbiological Examination of Foods (SMMEF), as reported by Vanderzant and Splittstoesser (1993). Malassezia isolates were identified according to the specification of Downes and Ito (2001). Antimicrobial Susceptibility test for bacterial isolates was conducted using standard disc diffusion method. Statistical analysis was carried out using SPSS version 17.

RESULTS

Table 1 shows the relative distribution of Gram positive bacterial isolates in fruits and vegetables. In fruits *Bacillus* species had the highest isolation rate of 44.0%, followed by *Staphylococcus aureus (38.3%).* The situation was reversal in vegetables where *Staphylococcus aureus* was predominant with a frequency of 38.3%, followed by *Bacillus species*. From the vegetables and fruits and *S. aureus* was isolated most from Slim banana (Fruit) and Onion (vegetable), *S. epidermidis* was isolated most from Orange (Fruit), Ugwu (Vegetable) and *Bacillus sp* was isolated most from grape and paw-paw (fruit) and cabbage and carrot (vegetables).

Table1: Distribution of gram positive bacteria in fresh Nigerian fruits and vegetables

Farm produce	*N*	S. aureus *n*	*(%)*	S. epidermidis *n*	*(%)*	Bacillus species *n*	*(%)*
Fruits							
African cherry	20	7	(35.0)	3	(15.0)	0	(0)
Fat banana	20	11	(55.0)	6	(30.0)	3	(15.0)
Slim banana	20	15	(75.0)	5	(25.0)	13	(65.0)
Grape	20	0	(0)	0	(0)	10	(50.0)
Lime	20	10	(50.0)	0	(0)	18	(80.0)
Mango	20	13	(65.0)	0	(0)	0	(0)
Orange	20	7	(35.0)	7	(35.0)	2	(10.0)
Paw-Paw	20	10	(50.0)	0	(0)	13	(65.0)
Pear	20	0	(0)	0	(0)	16	(80.0)
Pineapple	20	0	(0)	0	(0)	10	(50.0)
Tomato	20	8	(40.0)	0	(0)	12	(60.0)
Total	220	81	(36.8)	21	(9.6)	97	(44.0)
Vegetables							
Cabbage	20	0	(0)	0	(0)	10	(50.0)
Carrot	20	0	(0)	0	(0)	10	(50.0)
Cucumber	20	13	(65.0)	0	(0)	2	(10.0)
Garden Egg	20	7	(35.0)	7	(35.0)	7	(35.0)
Lettuce	20	0	(0)	0	(0)	0	(0)
Onion	20	15	(75.0)	5	(25.0)	0	(0)
Water Leaf	20	12	(60.0)	8	(40.0)	0	(0)
Water Mellon	20	12	(60.0)	4	(20.0)	16	(80.0)
Ugwu	20	10	(50.0)	10	(50.0)	0	(0)
Total	180	69	(38.3)	34	(18.9)	45	(25.0)

N= Total number of fruit/vegetable samples

n =Frequency of isolation from the fruit/vegetables samples

Table 2 demonstrates the frequency of Gram negative bacterial isolates in fruits and vegetables. *Klebsiella species* was the predominant isolate in fruits, recording a frequency of 40% while in vegetables, *Pseudomonas species* were prevalent, with a frequency of 14.4%. Only *Malassezia* and *Aspergillus species* were isolated as myco-flora of fruits and vegetables. In fruits, both *Malassiezia* and *Aspergillus species* had a close prevalence rates of 24.1% and 21.4% respectively while *Aspergillus species* were the predominant isolates in vegetables, with a frequency of 13.3% (Table 3).

Table 2: Gram negative bacteria isolated from fresh Nigerian fruits and vegetables.

Farm produce		*Klebsiella species*		*Proteus species*		*Pseudomonas species*		*Escherichia coli*	
	N	n	(%)	n	(%)	n	(%)	n	(%)
Fruits									
African cherry	20	7	(35.0)	3	(15.0)	3	(15.0)	3	(15.0)
Slim banana	20	9	(45.0)	3	(15.0)	0	(0)	3	(15.0)
Fat banana	20	18	(80.0)	9	(45.0)	0	(0)	0	(0)
Grape	20	0	(0)	0	(0)	0	(0)	0	(0)
Lime	20	16	(80.0)	0	(0)	0	(0)	0	(0)
Mango	20	0	(0)	0	(0)	8	(40.0)	8	(40.0)
Orange	20	12	(60.0)	0	(0)	2	(10.0)	2	(10.0)
Paw-paw	20	13	(65.0)	0	(0)	0	(0)	0	(0)
Pear	20	4	(20.0)	6	(30.0)	2	(10.0)	2	(10.0)
Pineapple	20	3	(15.0)	3	(15.0)	0	(0)	0	(0)
Tomato	20	6	(30.0)	0	(0)	0	(0)	0	(0)
Total	220	88	(40.0)	24	(10.9)	18	(8.2)	18	(8.2)
Vegetables									
Cabbage	20	0	(0)	0	(0)	5	(25.0)	0	(0)
Carrot	20	11	(55.0)	0	(0)	0	(0)	0	(0)
Cucumber	20	0	(0)	0	(0)	0	(0)	0	(0)
Garden Egg	20	0	(0)	0	(0)	0	(0)	0	(0)
Lettuce	20	0	(0)	0	(0)	0	(0)	0	(0)
Onion	20	0	(0)	0	(0)	0	(0)	0	(0)
Water leaf	20	0	(0)	0	(0)	0	(0)	0	(0)
Water melon	20	0	(0)	0	(0)	16	(80.0)	0	(0)
Ugwu	20	0	(0)	0	(0)	5	(25.0)	0	(0)
Total	180	11	(6.0)	0	(0)	26	(14.4)	0	(0.0)

N= Total number of fruit/vegetable samples
n =Frequency of isolation from the fruit/vegetables samples

Table 3: Fungi isolated from fresh Nigerian fruits and vegetables.

Farm Produce		*Malassezia species*		*Aspergillus species*	
	N	n	(%)	N	(%)
Fruits					
African cherry	20	13	(65.0)	7	(35.0)
Fat banana	20	0	(0)	3	(15.0)
Slim banana	20	0	(0)	0	(0)
Grape	20	17	(75.0)	3	(15.0)
Lime	20	10	(50.0)	10	(50.0)
Mango	20	0	(0)	4	(20.0)
Orange	20	0	(0)	0	(0)
Paw-Paw	20	0	(0)	0	(0)
Pear	20	10	(50.0)	10	(50.0)
Pineapple	20	3	(15.0)	10	(50.0)
Tomato	20	0	(0)	0	(0)
Total	220	53	(24.1)	47	(21.4)
Vegetables					
Cabbage	20	0	(0)	0	(0)
Carrot	20	0	(0)	5	(25.0)
Cucumber	20	0	(0)	0	(0)
Garden Egg	20	0	(0)	10	(50.0)
Lettuce	20	0	(0)	0	(0)
Onion	20	3	(15.0)	0	(0)
Water Leaf	20	0	(0)	0	(0)
Water Mellon	20	4	(20.0)	4	(20.0)
Ugwu	20	0	(0)	5	(25.0)
Total	180	7	(3.9)	24	(13.3)

N= Total number of fruit/vegetable samples

n =Frequency of isolation from the fruit/vegetables samples

Higher resistance was recorded against Cephalosporins (Ceftazidine =100%, Cefuroxine = 95.9%) than in the Quinolones (Table 4). In Table 5, a comparative analysis of mean zones of Ofloxacin inhibition against Gram positive bacterial isolates showed a significant difference ($F = 5.66$, $P<0.05$); with *Staphylococcus epidermidis* having the highest resistance to Ofloxacin. Among the Gram negative bacterial isolates, a significant difference was observed in the comparative mean zone of Pefloxacin inhibition ($F = 5.38$, $P<0.05$). Results further revealed that *Pseudomonas aeruginosa* was the most resistant isolate to Pefloxacin (Table 6).

Table 4: Antimicrobial resistance of fruits and vegetables.

Antibacterial agent	N	Resistance N	Percentage resistance (%)
Quinolones			
Ciprofloxacin (CIP)	532	6	(1.1)
Perfloxacin (PEF)	532	47	(3.8)
Ofloxacin (OFX)	532	29	(5.5)
Sperfloxacin (SPM)	532	141	(26.5)
Cephalosporin			
Cefuroxime (CXM)	532	510	(95.9)
Ceftazidime (CAZ)	532	532	(100.0)

N=number of bacteria challenged with antibiotic.

n =number of resistant bacteria.

Table 5: Comparative antibiotic susceptibility of gram positive bacteria isolated From fresh fruits and vegetables.

Antimicrobial Agents	*N*	*Bacterial Isolates*	*Zones of inhibition Mean ± SD*	*F*	*P– value*	*Most resistant isolate by LSD*
	150	*S. aureus*	24.23 ± 1.50			
Ciprofloxacin	55	*S. epidermidis*	22.88 ± 1.73	4.36	>0.05	
(CIP)	136	*Bacillus species*	22.30 ± 3.37			
	150	*S. aureus*	19.20 ± 4.33			
Pefloxacin	55	*S. epidermidis*	19.88 ± 4.33	2.37	>0.05	
(PEF)	136	*Bacillus species*	16.00 ± 5.94			
	150	*S. aureus*	20.14 ± 5.67			
Sperfloxacin	55	*S. epidermidis*	17.75 ± 3.20	1.51	>0.05	
(SPM)	136	*Bacillus species*	22.80 ± 9.09			
	150	*S. Aureus*	23.97 ± 4.22			
Ofloxacin	55	*S. epidermidis*	19.88 ± 3.52	5.66	<0.05	*Staph.*
(OFX)	136	*Bacillus species*	20.30 ± 3.23			*epidermidis*
	150	*S. aureus*	7.60±3.06			
Cefuroxime	55	*S. epidermidis*	0.00 ± 0.00	1.41	>0.05	
(CXM)	136	*Bacillus species*				
	150	*S. Aureus*	0.00 ± 0.00			
Ceftazidime	55	*S. epidermidis*	0.00 ± 0.00	-		
(CAZ)	136	*Bacillus species*	0.00 ± 0.00			

N = number of resistant bacterial isolates
LSD= Least significant difference

Table 6: Comparative antibiotic susceptibility of gram negative bacteria isolated from

Antimicrobial Agents	*N*	*Bacterial Isolates*	*Zones of inhibition Mean ± SD*	*F*	*P– value*	*Most resistant isolate by LSD*
	28	*Proteus species*	25.50 ± 3.31			
Ciprofloxacin	10	*Escherichia coli*	31.33 ± 0.58			
(CIP)	51	*Pseudomonas*	26.50 ± 0.71	2.79	>0.05	
	10	*spp.*	28.88 ± 4.15			
	9	*Klebsiella spp.*				
	28	*Proteus species*	19.40 ± 3.10			
Pefloxacin	10	*Escherichia coli*	25.67 ± 4.04			*Pseudomonas*
(PEF)	51	*Pseudomonas*	18.50 ± 0.71	5.38	<0.05	*Species*
	10	*spp.*	18.88 ± 2.58			
	9	*Klebsiella spp.*				
	28	*Proteus species*	18.00 ± 1.83			
Spefloxacin	10	*Escherichia coli*	25.00 ± 3.46			
(SPM)	51	*Pseudomonas*	18.50 ± 4.95	1.50	>0.05	
	10	*spp.*	19.63 ± 5.97			
	9	*Klebsiella spp.*				
	28	*Proteus species*	27.50 ± 0.71			
Ofloxacin	10	*Escherichia coli*	24.67 ± 0.71	1.86	>0.05	
(OFX)	51	*Pseudomonas*	23.00 ± 1.41			
	10	*spp.*	23.88 ± 4.54			
	9	*Klebsiella spp.*				
	28	*Proteus species*	0.00± 0.00			
Cefuroxine	10	*Escherichia coli*	0.00± 0.00	2.19	>0.05	
(CXM)	51	*Pseudomonas*	0.00 ± 0.00			
	10	*spp.*	1.59 ± 3.78			
	9	*Klebsiella spp.*				
	28	*Proteus species*	0.00 ± 0.00			
Ceftazidime	10	*Escherichia coli*	0.00 ± 0.00	0.84	>0.05	
(CAZ)	51	*Pseudomonas sp*	0.00 ± 0.00			
	10	*Klebsiella spp.*	1.42 ± 3.35			
	9					

N = number of resistant bacterial isolates
LSD= Least significant difference

DISCUSSION

The number of documented outbreaks of human infections associated with the consumption of raw fruits and vegetables has increased in recent years (Akhtar *et al*., 2014; Uyttendaele *et al*., 2014). In this study, higher microbial contamination has been noticed in fat banana and water melon. This contamination may however not have serious consequences if the skins or rinds were to be peeled off before consumption. The microorganisnms present in fruits and vegetables as indicated in this study may be a direct reflection of the poor hygienic production and transport facilities.

The isolation of bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus species, Escherichia coli, Proteus species, Pseudomonas species, Klebsiella species,* and fungi such as *Malassezia species* and *Aspergillus species* shows that fruits could act as a medium for the spread of both pathogenic and opportunistic microbes. These organisms isolated are also similar to those isolated by Mathur *et al*., 2014; Ankita, 2010; Eni *et al*., 2010. Isolation of *Escherichia coli* (coliforms) and enteric bacteria (*Klebsiella species and Proteus species*) from fruits & vegetables suggested their likely-hood of faecal contamination (Issa-Zacharia *et al*., 2010), they could also arise from the use of human manure. Also, isolation of environmental isolates such as *Pseudomonas, Bacillus* and *Aspergillus species*, may be indicative of soil contamination. The isolation of skin commensals such *Staphylococcus epidermidis, Staphylococcus aureus and Malassezia species* (Abdelnoor *et al*., 1983), may serve as indicator of contamination by handlers, either during transportation or post-harvest processing. The resistance of isolated bacteria to Quinolones in this study was generally low, though a relatively higher resistance of 26.5% was recorded against Sperfloxacin, the reasons for this resistance to Sperfloxacin is unexplainable. Relative degree of resistance demonstrated by *Staphylococcus epidermidis* against Ofloxacin and *Pseudomonas species* against Pefloxacin has re-affirmed the report of Eni *et al* (2010).Bacterial isolates of fruits and vegetables from this study has also demonstrated an absolute resistance to Ceftazidine (100%) and Cefuroxime (95.9%). The implication of this is that these drugs would be of no benefit in the treatment of infections caused by any of the two micro-floras. It's a bit difficult to conclude on the origin of antibacterial resistance especially, those against Cephalosporins (i.e the Extended Spectrum Beta Lactam drugs). The prevalence of antibiotic resistant microbes in fruits and vegetables constitutes a great health risk in developing countries (Razzaq *et al*., 2014).

From our observations, it is evident that fruits and vegetables could act as a potential vehicle for the dissemination of antibiotic resistance, as well as medically important microorganisms. Future study should therefore be undertaken at molecular level in order to establish whether the antibiotic resistance in these floras of fruits and vegetables are plasmid-borne or not. And if the resistance gene is plasmid-borne, an attempt should therefore be made to establish whether the genes are transferable or not. The outcome of further studies will go a long way in answering questions on how antibiotic resistant genes may be transferred from micro flora of fruits and vegetables to the human intestinal flora, after consumption.

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