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Research

Incidence of Cephalosporin Resistant Bacterial Flora in Fruits and Vegetables Purchased in Ilishan Markets

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Abstract

Raw and minimally processed fruits and vegetables are essential part of peoples' diet around the globe. These agricultural products may, however, serve as vehicle of drug-resistant human diseases. Hence, this study was, therefore, carried out to determine the antibiotic resistance profile of bacterial flora from fruits and vegetables purchased in some market locations in Ilishan community. Swabs from the surfaces of fruits and vegetables were aseptically inoculated and serially diluted in 0.1% peptone water. Bacterial total count (TC) and enterobacteriaceae count (EC) were determined on nutrient agar and MacConkey agar respectively, after aerobic incubation at 37°C for 48hours. Speciation of bacterial isolates was determined by standard microbiological techniques. Susceptibility of the isolated bacteria against some selected cephalosporins and quinolones was determined by agar-disc diffusion method. The most frequently isolated organisms were Klebsiella species (41.8%), followed by Pseudomonas aeroginosa (26.1%) while Salmonella species (2.2%) and Candida albicans was least with a frequency of 2.2%. Higher frequency of antibacterial resistance was recorded against augmentin (86.7%) and cefuroxime (84.4%), with a record of 64.4% multiple resistance against both drugs. From this study it was established that fruits and vegetables can serve as potential media for the dissemination of both enteric and non-enteric microbial pathogens, including bacterial strains that exhibit multiple resistance to the cephalosporins, in the human community.

KEY WORDS:

INTRODUCTION

Fruits and vegetables are important component of our daily diet. They contain vitamins and minerals and plant chemicals called phytochemicals which are bioactive nutrients or compounds in fruits, vegetables, grains and other plant that have been linked to reductions in the risks of major chronic diseases (Adams *et al.*, 2004). Regular consumption of fruit is associated with reduced risks of cancer, cardiovascular disease, stroke, Alzheimer disease, cataracts, and some of the functional declines associated with ageing (Booth *et al.*, 1999). Diets that include a sufficient amount of potassium from fruits and vegetables also help reduce the chances of developing kidney stones and may help reduce the effects of bone-loss (Beuchat, 1996b). Fruits are also low in calories which would help lower one's calorie intake as part of a weight-loss diet (Adams *et al*., 2004). Fruits and vegetables can help to protect the body against some diseases including diabetes, stroke, some cancers and hypertension (Cothran *et al*., 2003).

Raw and minimally-processed fruits and vegetables are essential part of peoples's diet around the globe. Where land is available, families grow fruits and vegetables using human and animal excreta as fertilizers. Alternatively, fruits and vegetables are purchased from local farms or retail outlets for further preparation by street vendors, by families at home or as part of meal eaten in restaurants and other food-services facilities.

The surface flora on fresh produce may reflect the environmental flora where the products are grown. Contamination of the products may take place at all stages, including pre- and post- harvests and the processing stage (Beuchat, 1997; De Roever, 1999). Possible sources of contamination are soil, faeces, manure of human or animal origin, water for irrigation, animals including insects and birds, handling of products, harvesting and processing equipment and transport (Gross *et al*., 2002).

In light of the above, there have been suggestions that contaminated fruits and vegetables may serve as vehicles of human diseases (Beuchat, 1996b; PAHO/NNPPAZ, 1996). However, these claims cannot be generalized due to inadequate documentation. A wide range of enteric pathogens and their toxins can be transmitted through food. These include the bacteria such as *Campylobacter* species, *Salmonella* species, *Shigella* species, entero-virulent *Escherichia coli*, *Clostridium botulinum, Listeria monocytogenes*, *Clostridium perfringens*, some *Bacillus* species, *Staphylococcus aureus*, the protozoa such as *Cryptospordium parvum*, *Cyclospora cyatenenesis* and *Giardia* species, and viruses such as Norwalk-like viruses and Hepatitis A (Lewis *et al*., 2002).

Spread of microbial resistance to antibiotics and its associated drug-resistant ailments, is a global threat to the entire human race. In order to curtail this menace, intensive investigations are continuously being carried out by researchers all over the world so as to uncover all the available means through which resistant organisms are acquired by humans and how these can best be prevented. Although, it is well established that food may be a source of pathogenic bacteria to humans however, little or none is known about the normal flora of vegetables and in particular, their resistance to antibiotics as was recently reported to the United Kingdom Government (Advisory Comm. of Microbiology, 1999).

Besides the indigenes, Ilishan community is largely populated by Babcock staff and students some of whom are vegetarians and therefore, depends on fruit and vegetable diets for their livelihood. Furthermore, there is dearth of information on the possible role of fruits and vegetables as sources for the dissemination of antibiotic resistant pathogens among humans in Ilishan community.

AIM AND OBJECTIVES

Hence, this study was carried out to:

1. Determine the microbiological safety of the fruits and vegetables, purchased from Ilishan community markets.
2. Determine the antibiotic resistance profile of bacterial flora of fruits and vegetables, purchased in Ilishan community.
3. Evaluate which among the fruits and vegetables harbours the highest total bacterial and enterobacteriaceae density.
4. Assess the antibiotic resistance pattern and profile of the isolated bacteria.

MATERIALS AND METHODS

Sample collection

Fruit and vegetable samples were separately bought from five sellers in different market locations in Ilishan community. Each of the sample group comprises of 5 varieties from individual sellers. Forty (i.e 8) types of fruits and ten (i.e 2) types of vegetables were purchased from Ilishan community markets. The vegetables are onion and carrot while the fruits are orange, pineapple, pawpaw, apple, banana, tangerine, water melon and tomato.

Each batch of samples from the sellers was separately collected into well labelled, pre-sterilized, stainless steel containers with lids. The samples were immediately transported to the laboratory after collection. While in the laboratory, pairs of latex gloves were worn prior to sample selection and processing.

Sampling

A total of five batches of individual sample (i.e fruits and vegetables), were obtained from five sellers in Ilishan community. One random sample was selected from each batch to constitute a sample group (n=5) for each fruit and vegetable. Each randomly selected sample from the sellers was assigned a serial code e.g (P_1, P_2, P_3, P_4 and P_5).

Sample analysis

After moistening of a wooden swab stick with sterile nutrient broth, the entire surface of each fruit and vegetable was carefully swabbed. The swab was aseptically transferred into a sterile 0.1% nutrient broth, the wooden stem was broken off and the bottle was screw-capped and labelled with the sample code. The sample bottle was vigorously shaken and tapped with finger for a period of 30 seconds to 1 minute in order to ensure that all the attached bacterial cells were dislodged from the absorbent cotton swab. The sample was then left undisturbed for a period of 1hour.

CULTURE AND PLATE COUNT

Miles and Misra

The samples (inoculated 0.1% nutrient broth) were serially diluted tenfold (from 10^1-10^{10}). A grease pencil was used to demarcate a well dried nutrient agar (for total count) and MacConkey agar (for Enterobacteriaceae count) into segments. With the aid of pre-sterilized 50 dropper Pasteur pipettes, a drop of each dilution was inoculated into each corresponding segment. The plate was covered with lid, left on the bench for a period of 30minutes for adequate absorption of the inoculated sample. These were incubated aerobically for 24hours at 37°C.

Note: the plate counts were prepared in duplicates. After incubation, the colonies of the isolated bacteria in each segment were counted (especially segments containing 20-200 colonies). The colonies of the two plates were counted and the average taken as the bacterial count for the specific segment.

Culture

The first (1/10) dilution was aseptically inoculated and streaked on blood agar and MacConkey agar using pre-sterilized wire loop. The plates were incubated aerobically at 37°C for 24 hours.

Identification of organisms

The identities of the isolated organisms were confirmed by Gram staining, spore staining and biochemical tests while antimicrobial susceptibility test was also carried out by standard disc-agar diffusion technique as specified by the National Committee on Clinical Laboratory Standard (NCCLS) (Ochei and Kolhatkar, 2007).

Results

Table 1 showed that out of the 50 vegetables and fruits, screened for the presence of microflora, 42(84.0%) harboured microorganisms while 8(16.0%) showed no growth of microorganisms.

Table 1: Number of fruits and vegetables purchased from Ilishan community markets.

	No of samples with microflora	
Samples	**N**	**n**
Fruits		
Orange	5	5
Pine apple	5	5
Paw paw	5	3
Apple	5	3
Banana	5	4
Tangerine	5	5

Water melon	5	4
Tomato	5	4
Vegetables		
Onion	5	5
Carrot	5	4
Total	**50**	**42**

The mean total bacteria counts from each of the samples were compared in table 2. Result showed no significant difference in the bacterial densities of the samples **(F= 1.77, P>0.05).**

Table 2: Comparison of total bacterial count among fruits and vegetables purchased from Ilishan community markets.

Samples	n	Total count (CFU/ml) mean ± SEM	High bacterial density
Water melon	**5**	23000.00±19339.08	None
Orange	**5**	155900.00±79567.96	
Pine apple	**5**	156300.00±70172.57	
Tangerine	**5**	205000.00±88175.96	
Tomato	**5**	207100.00±9744.10	
Onion	**5**	210000.00±29566.60	
Banana	**5**	242000.00±11675.90	
Apple	**5**	245000.00±40323.60	
Carrot	**5**	262000.00±11103.60	
Paw paw	**5**	1333000.00±817917.50	

F= 1.77, P > 0.05

In table 3, the mean Enterobacteriaceae count (CFU/ml) of each of the fruit and vegetable were compared. A significant difference was observed in the Enterobacteriaceae density in which the highest count was recorded in orange, followed by pine apple, onion and paw paw **(F= 2.87, P<0.05)**.

Table3: Comparison of Enterobacteriaceae count among fruits and vegetables purchased in Ilishan community.

Samples	n	Enterobacteriaceae count (CFU/ml) mean ± SEM	Highest bacterial density
Orange	**5**	36700.00±9559.81	Orange
Pine apple	**5**	22000.00±9027.74	Pine apple
Banana	**5**	1000.00 ±1000.00	Onion
Paw paw	**5**	20100.00 ± 258.77	Paw paw
Tomato	**5**	1600.00 ± 600.00	
Onion	**5**	21800.00±15826.09	
Apple	**5**	3500.00 ± 955.76	
Water melon	**5**	1500.00 ± 707.11	
Tangerine	**5**	4000.00 ± 2792.85	
Carrot	**5**	1000.00 ± 1000.00	

F= 2.87, P<0.05

Fruits and vegetables were assesed for their microbiological safety based on acceptable reference limit for both total count (i.e <10^6 organisms/ml) and Enterobacteriaceae count (i.e <10 organisms/ml). Out of 50 samples, 19 (38.0%) of them were safe for consumption while 31 (62.0%) were unsafe for consumption (Table 4).

Table 4: Assessment of microbiological safety of fruits and vegetables purchased from Ilishan community.

Fruits & vegetables (Samples)	n	(%)
Accceptable	19	(38.0)
Not acceptable	31	(62.0)
Total	**50**	**(100.0)**

In table 5, frequency distribution of microflora from the analyzed fruits and vegetables were showed. *Klebsiella* species recorded the highest isolate of 41.8% followed by *Pseudomonas aeroginosa* with a frequency of 26.1% while the least frequency were both displayed by *Salmonella* species and *Candida albicans* with each recording a frequency of 2.2%.

Table 5: Frequency distribution of microflora from fruits and vegetables purchased in Ilishan community.

Microflora	n	(%)
Klebsiella species	19	(41.8)
Pseudomonas aeroginosa	12	(26.1)
Providencia species	4	(8.7)
Bacillus subtilis	3	(6.5)
Escherichia coli	3	(6.5)
Staphylococcus epidermidis	3	(6.5)
Salmonella species	1	(2.2)
Candida albicans	1	(2.2)
Total	**46**	**(100.0)**

Table 6 displayed the frequency of antibacterial resistance from bacterial flora of fruits and vegetables in which highest frequency of resistance (86.7%) was recorded against Augmentin followed by cefuroxime with a frequency of 84.4% while no resistance (100% susceptibility) was recorded against ciprofloxacin.

Table 6: Distribution of antibacterial resistance among the bacteria flora from fruit and vegetables purchased in Ilishan community markets.

Antibacterial agents	N	n	(%)
Augmentin (AUG)	45	39	(86.7)
Cefuroxime (CXM)	45	38	(84.4)
Ceftazidime (CAZ)	45	10	(22.2)
Pefloxacin (PEF)	45	1	(2.2)
Ofloxacin (OFX)	45	1	(2.2)
Ciprofloxacin (CIP)	45	0	(0)

In table 7, distribution of multiple antibacterial resistance profile of the bacterial flora of fruits and vegetables from Ilishan community market was displayed in table 7. The bacterial flora exhibited multiple drug resistance against Augmentin and Cefuroxime, with a frequency of 64.4%. This was followed by multi-drug resistance against Augmentin, Ceftazidime and Cefuroxime with a frequency of 22.2%.

Table 7: Distribution of multiple antibacterial resistance profile of bacterial flora isolated from fruit and vegetables in Ilishan community markets.

Profile of Antibacterial agent	n	(%)
AUG/CXM	29	(64.4)
AUG/CAZ/CXM	10	(22.2)
AUG/CAZ/CXM/PEF	3	(6.7)
CXM	3	(6.7)
Total	**45**	**(100.0)**

DISCUSSION, CONCLUSION AND RECOMM-ENDATION

In developing countries, food-borne illness caused by contaminated fruits and vegetables are frequent and in some areas, they cause, a large proportion of illnesses (PAHO/NNPPAZ, 1996). Over the past decades, there has been an increase in per capita consumption of fresh fruits and vegetables (Beuchat *et al*., 2001) because of increased awareness in healthy diet. However, life-threatening diseases and infections have been reportedly linked with fruit and vegetable microflora and fruits contaminating pathogens (Beuchat *et al*., 1998).

In this study, out of the 50 fruits and vegetables, purchased in Ilishan community, the rate of microflora on the surface of fruits and vegetables sold in Ilishan community was about 84.0%. This high figure is in conformity with the hypothesis that microorganisms are ubiquitous, and that they could be contacted from different sources. Dowe et al., (1997); Lund, (1992); Beuchat, (1995) and NACMCF, (1999) reported that pre-and post-harvest contamination of fruits and vegetables may occur from use of human faeces as manure, irrigation of plants with dirty water, contamination from pests and human handlers.

Although, the total bacterial counts (CFU/ml) among the fruits and vegetables were at equal densities (P>0.05), higher densities of enterobacteriaceae counts (CFU/ml) were observed in orange, pine apple, onion and paw paw (P<0.05). This implies that the aforementioned fruits and vegetables are more prone to faecal contamination. Although, it may be a bit difficult to explain the rationale behind higher densities of faecal bacteria in some fruits and vegetables as compared to others, since there was no informative data on the hygienic standards of the sellers. Equilibrium in the total bacterial density; suggesting equal degree of bacterial contamination tends to link the occurrence of faecal bacteria on surfaces of the fruits and vegetables with their poor treatment. Since the faecal pathogens are rarely isolated from their environment (food, water, etc) due to their low viability outside the gut, hence, observation of the faecal bacteria in fruit and vegetable samples is suggestive that the samples are subject to contamination by faecal pathogens such as *Salmonella*, *Shigella*, *Clostridium* etc. Pre- and post-harvest contamination of fruits and vegetables have been reportedly linked with animal manure, handling with contaminated hands and treatment with contaminated water Dowe *et al*., (1997) and Lund, (1992). Microbiological assessment of the fruit and vegetable samples revealed that 62.0% of the samples were not safe for consumption. This high rate of unsafe fruit and vegetable samples potrays an imminent danger for the populace who are the consumers of these agricultural products.

Different types of microflora were isolated from the surface of the fruits and vegetables. The isolated bacterial flora are *Klebsiella* species, *Pseudomonas aeroginosa*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Providencia* species and *Salmonella* species while the only isolated mycoflora was *Candida albicans*. *Klebsiella* species followed by *Pseudomonas aeroginosa*, were the two predominant bacteria agents in the samples having recorded the frequency of 41.8% and 26.1%

Beuchat L.R., Nail B.V., Alder B.B. and Clavero M.R.S (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria

respectively. The isolated microflora were similar to those reported by Komines *et al.*,(1972) and Beuchat, (1995), but differ in the sense that *Salmonella* species was isolated.

The isolated bacteria demonstrated a high degree of resistance against cephalosporins, especially Augmentin (AMC) and Cefuroxime (CXM). Although, double-disc synergy test (DDST) could not be conducted in order to ascertain the Extended Spectrum Beta Lactamase (ESBLS) -producing status of the isolated organisms. Majority of the isolates demonstrated multiple-drug resistance of which the highest frequency was recorded against Augmentin (AMC) and Cefuroxime (CXM).

In conclusion, this study has established that fruits and vegetables can serve as potential sources for carriage and transfer of both enteric and non-enteric microbial pathogens, including bacterial strains with multiple resistance to cephalosporins. Based on the high density of enteriobacteriaceae in the studied fruits and vegetables, it is hereby suggested that the use faecal manure by Ilishan farmers be discouraged.

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