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Microbiological Studies of Vegetable Leaves Sold in Elele Market, Rivers-State, Nigeria

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Abstract

The present study was carried out to evaluate some vegetable leaves sold in Elele markets for human consumption by investigating whether they harbour bacteria. Out of 300 samples examined, 218 (72.6%) harboured bacterial isolates. Depending on the types of vegetable leaves analysed, water leaves recorded the highest frequency of infected samples (16.0%), following by pumpkin leaves (15.0%), while bitter leaves had the lowest by (13.3%). Species of bacteria isolated were: *Escherichia coli* (29.3%), *Staphylococcs aureus* (22.9%), *Enterobacter aerogenes* (18.3%), *Pseudomonas aeruginosa* (8.2%), *Shigella* species (5.5%), *Alcaligenes feacalis* (4.6%), *Micrococcus* species (3.7%) and *Salmonella* species (2.1%). All samples of water leaves, pumpkin leaves and green leaves harboured ten different bacterial species, with water leaves samples recording the highest occurrence of *Escherichia coli* (39.6%), *Staphylococcus aureus* (16.8%), *Enterobacter aerogenes* (15.4%), *Pseudomonas aeruginosa* (11.4%), followed by pumpkin leaves with *Escherichia coli* (29.7%), *Staphylococcus aureus* (18.1%), *Pseudomonas aeruginosa* (12.9%). In contrast, *Citrobacter* species, *Micrococcus* species, *Pseudomonas aeruginosa* and *Salmonella* species could not be isolated from bitter leaves samples. The results of antibiotic susceptibility showed that Gram negative bacteria were highly sensitive (58.5-100%) to ofloxacin, (55.6-100%) to ciprofloxacin, while most of them were less sensitive (0.0-22.7%) to tetracycline. Gram positive bacteria had a susceptibility (90.9-100%) to pefloxacin, (81.8-100%) to ciprofloxacin and *Bacillus cereus* recorded the least susceptibility (9.1%) to erythromycin. Findings in this study still indicate poor handling practices and risks presented to the consumers at point-of-sale. There is need to educate the vendors and consumers on good sanitary practices during processing, display and sale of vegetables and also dangers associated with misuse of antibiotics. Quinolones could be the drugs of choice in the treatment of bacteria associated with food-borne infection.

Keywords: Elele; Bacteria; Vegetables; Antibiotics susceptibility

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Introduction

Fresh vegetables are regarded as important part of a healthy diet. In many countries, such leafy plants are eaten raw or lightly cooked to preserve taste and this practice may also favour the likelihood of food-borne infections (Ozlem and Sener) [1]. Vegetables can be contaminated with pathogenic microorganisms during harvesting through faeces, sewages, untreated irrigation water or surface water (Feng et al.) [2]. Also, unsafe water used for rinsing the vegetables an sprinkling to keep them fresh is also a source of contamination (Froder et al.) [3]. In recent years, there has been an increase in the number of reported cases of food-borne illness linked to the consumption of fresh vegetables. Sickness related to food-borne infection transcends all geographical, political and cultural boundaries. The incidence of food-borne diseases continues to adversely affect the health and productivity of populations of most countries, especially non-industrialized ones (Northo-Clews and Shaw) [4]. It has been estimated that about 20% of all vegetables harvested for human consumption are lost

through microbial spoilage (Jay). The most common bacteria related to vegetable spoilage are: *Bacillus* cereus, *Micrococcus* species, *Staphylococcus aureus*, Staphylococcus epidermidis, *Escherichia coli*, Proteus mirabilis and *Enterobacter aerogenes* (Harrigen and McCance) [5]. Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable borne-out break that occurred in Japan in 1996 in which 11,000 people were affected and about 6,000 cultures were confirmed. Tambekar and Mundhada in 2006, examined salad vegetables in Amravati city and reported the presence of *Escherichia coli* (38.3%), *Enterobacter aerogenes* (20.9%), *Pseudomonas* species (16.2%), *Staphylococcus aureus* (15.1%), *Salmonella* species (5.8%) and *Shigella* species (3.4%). Ankita Rajvanshi analysed salad vegetables sold in Jaipur city, India and reported the presence of *Bacillus* species (24,5%), *Escherichia coli* (11.8%), *Pseudomonas* species (9.0%) *Streptococcus* species (6.4%), *Klebsiella* species (5.4%) and *Citrobacter* species [6]. In Nigeria, it has been noticed that local farmers make use of waste and untreated discharged water in Elele and environs for irrigation and washing which is suspected to be the primary source of contamination of microorganisms in vegetables. Hence, a sanitary survey was carried out to examine bacterial flora present on vegetable leaves sold in local markets in Elele, Rivers – State Nigeria.

Materials and Methods

Sample collection and processing

A total of 300 vegetables leaves samples made up of 60 pumpkin leaves (*Telfairi occidentallis*), 60 Okazi leaves (Genetum gnecanum), 60 water leaves (Talilum triangulare), 60 green leaves (Celosta argentea) and 60 bitter leaves (Vernonia amygdalina) were bought randomly in Elele market in Rivers – State, Nigeria from January 2015 to September 2015. About 50 g of each vegetable sample purchased was sliced in pieces and packaged into plastic bags, rinsed in 250 ml beaker containing 150 ml of physiological saline solution (0.95% Nacl). Rinsed water samples were diluted to 10^{-2} and 10^{-5}. About 0.1 ml from each dilution was plated on Mac-Conkey agar (Oxoid), SS (*Salmonella* and *Shigella*) agar (Oxoid), *Bacillus cereus* agar (Oxoid) and incubated at 37°C for 24 hours. The plates were sub-cultured and maintained on nutrient agar. The bacterial isolates were identified on the basis of morphological, cultural and biochemical tests (Cheesbrough).

Susceptibility testing

Disc diffusion antimicrobial susceptibility of the isolates was tested by agar disc diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standard Institute (CLSI) guide lines (2007). The following antibiotics were used: ampicillin (25 µg) chloramphenicol (30 µg), gentamicin (10 µg), cephalecin (15 µg), tetracycline (25 µg), ciprofloxacin (10 µg), cotrimoxazole (30 µg), erythromycin (10 µg), amoxicillin (30 µg), streptomycin (10 µg), and zinnat (20 µg).

Results

Frequency of distribution of some infected vegetable leaves sold in Elele market is shown in **Table 1**. Out of 300 vegetable leaves examined, 218 (72.6%) harbored bacterial isolates. Water leaves had the highest number of infected samples 48 (16.0%) followed by pumpkin leaves 45 (15.0%), while bitter leaves recorded the lowest 40 (13.3%). Ten species of bacteria were isolated as shown in **Table 2**; with *Escherichia coli* being the most predominant 128 (29.3%), followed by *Staphylococcus aureus* 100 (22.9%), *Enterobacter aerogenes* 80 (18.3%). *Salmonella* species was the least 9 (2.1%) **(Tables 1 and 2)**.

Table 3 shows the distribution of bacteria isolated from vegetable samples. All the ten bacterial species were isolated from water leaves, pumpkin leaves and green leaves. Water leaves recorded the highest number of *Escherichia coli* 59 (39.6%), *Staphylococcus aureus* 25 (16.8%), *Enterobacter aerogenes* 23 (15.4%), *Pseudomonas aeruginosa* 13 (12.9%). While *Citrobacter* species, *Micrococcus* species, *Pseudomonas aeruginosa* and *Salmonella* species could not be isolated from bitter leaves samples **(Table 3)**.

Tables 4a and 4b show the antibiotic susceptibility patterns of the bacterial isolates. Most of the Gram negative bacteria were susceptible to quinolones, with the susceptibility range from 58.3-100% to ofloxacin; 55.6-100% to ciprofloxacin. While *Bacillus cereus* recorded the least with 1 (9.1%) against pefloxacin.

Discussion

Reports of cases of gastroenteritis following consumption of meals served with fresh vegetables have become a serious public health concern. In developing countries, there are serious concerns about the sanitation of fresh vegetables, especially in most restaurants where dishes and utensils, are often washed with single change of water in the same bowl all day long. Deterioration of fresh vegetables can result from physiological breakdown due to physical damage or invasion by microorganisms (Wilson) [7]. Microbiological quality of fresh vegetables was examined in Elele community, Rivers-State. Out of 300 vegetable samples analysed, about 218 (72.6%) yielded growth of bacterial isolates and this was in accordance with the reports of Ankita (2010) [8]. This high level of bacterial contamination is due to factors such as inability to observe the basic sanitation during consumption, non-availability of water in good quality for washing and pre-disinfection of fresh vegetables during mass production added to this are poor personal handling from the period of harvest, transportation, storage and most especially during exposure in the markets for sales and reckless handling from person to person. Water leaves recorded the highest level of infected samples (80.0%), followed by pumpkin leaves (75.0%); this is attributed to the mode of propagation of these vegetables during farming on the wet, humid and contaminated soil. Both Gram negative and Gram positive belonging to ten genera were identified. Among which, about seventy percent consists of enterobacteriaceae such as *Escherichia coli* (29.3%), *Enterobacter aerogenes* (18.3%), *Pseudomonas aeruginosa* (8.2%), *Shigella* species (5.5%), *Alcaligenes faecalis* (4.6%), *Citrobacter* species (2.9%) and *Salmonella* species (2.1%). The Gram positive bacteria included: *Staphylococcus aureus* (22.9%), *Micrococcus* species (3.7%) and *Bacillus cereus* (2.5%). Some of these isolates (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) were reported isolated from vegetables in Zaria (Gbonjubola) [9].

Table 1 Distribution of infected vegetable leaves sold in Elele market, Rivers State, Nigeria.

Vegetable	*E. coli*	*Enterobacter aerogenes*	*A. faecalis*	*Citrobacter* species	*Bacillus cereus*	*Micrococcus* species	*P. aeruginosa*	*Shigella* species	*Salmonella* species	*Staph. aureus*
Pumpkin leaves (*T. occidentallis*)	30 (29.7)	15 (14.9)	5 (4.9)	2 (1.9)	3 (2.9)	5 (4.9)	13 (12.9)	7 (6.9)	2 (1.9)	19 (18.1)
Okazi leaves (*G. gnecanum*)	16 (32.0)	10 (20.0)	0 (0.0)	2 (4.0)	1 (2.0)	2 (4.0)	2 (4.0)	2 (4.0)	0 (0.0)	15 (30.0)
Green leaves (*C. argenlea*)	13 (18.0)	17 (23.6)	4 (5.6)	3 (4.2)	2 (2.8)	3 (4.2)	4 (5.6)	6 (8.3)	3 (4.2)	17 (23.6)
Water leaves (*T!. triangulare*)	59-39.8	23 (15.4)	3 (2.0)	6 (4.0)	2 (1.3)	5 (3.6)	17 (11.4)	5 (3.6)	4 (2.6)	25 (16.8)
Bitter leaves *V. amygdalina*	10-18.2	15 (27.3)	8 (14.5)	0 (0.0)	3 (5.5)	0 (0.0)	0 (0.0)	4 (7.3)	0 (0.0)	15 (27.3)

Table 2 Percentage of occurrence of bacteria in vegetable leaves sold in Elele market, Rivers State, Nigeria.

Vegetable	No. of vegetables Examined	No. of vegetable Samples positive (%)
Pumpkin leaves (*T. occidentallis*)	60	45(15.0)
Okazi leaves (*G. gnecanum*)	60	42(14.0)
Green leaves (*C. argentea*)	60	43(41.3)
Water leaves (*T!. triangulare*)	60	48(16.0)
Bitter leaves (*V. amygdalina*)	60	40(13.3)
Total	300	218(72.6)

T: Telfairi; G: Genetum; C: Celosta; T!: Talilum; V: Vernonia

Table 3 Distribution of bacteria isolated from different vegetable leaves sold in Elele market, Rivers State, Nigeria (%).

Bacteria	No. of bacterial isolates	Occurrence (%)
Escherichia coli	128	29.3
Enterobacter	80	18.3
Citrobacter species	13	2.9
Alcaligenes faecalis	20	4.6
Pseudomonas aeruginosa	36	8.2
Salmonella species	9	2.1
Shigella species	24	5.5
Bacillus species	11	2.5
Micrococcus species	16	3.7
Staphylococcus aureus	100	22.9
Total	437	100

A: *Alcaligenes*; E: *Escherichia*; P: *Pseudomonas*; Staph: *Staphylococcus*; T: *Telfairi*; G: *Genetum*; C: *Celosta*; T!: *Talilum*; V: *Vernonia.*

Bacteria belonging to the same genera were also identified from fruits and vegetables analysed in Sango, Ota, Nigeria (Eni and Solomon) [10]. In India, most of those bacteria were grown from vegetables and fruits (Tambekar and Mundhada) [11]. The high percentage of occurrence of *Escherichia coli* (29.3%) could be attributed to the fact that; water is a major means by which *Escherichia coli* spread and the use of facially contaminated water for irrigation and washing of vegetables in a problem. *Staphylococcus aureus* was the second most predominant bacteria (22.9%), which is of great concern and could be due to its carriage in nasal passages of vegetable handlers or by infected workers (Beuchat) [12]. *Staphylococcus aureus* has been reported to remain the most prominent etiology of pyogenic infections and that staphylococcal infection leads to a worsening of some already existing superficial infections (Adegoke and komolafe) [13]. The contamination by *Salmonella* species and *Shigella* species of the vegetables is due to the handling of vegetables by infected workers, vendors and consumers in market place which help to spread pathogenic microorganisms, and also washing of vegetables with contaminated water. Increasing media coverage has focused on vegetable product as source of many *Salmonella* outbreaks.

Micrococcus species and *Bacillus cereus* were not isolated from all the vegetable samples and their presence rendered those vegetables unsatisfactory. Soil is one of the most important reservoir of many microbes and also contributes to the increased contamination and the presence of bacteria such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Citrobacter* species and *Alcaligenes faecalis* on the vegetables occurs when there is relax in hygiene standard.

The susceptibility testing of the isolates to some commonly prescribed antibiotics revealed multidrug resistant strains among the isolates. *Escherichia coli* exhibited significant level of multidrug resistance. Being one of the most important gastrointestinal flora capable of accepting and transferring plasmids and these plasmids can be transferred readily under stress to other species. This attribute has made *E. coli* to be considered as an important reservoir of transferable antibiotic resistance (Albinu et al.) [14]. The multidrug resistance ability demonstrated by *Pseudomonas aeruginosa* in this work becomes questionable as *Pseudomonas aeruginosa* is a frequent cause of nosocomial infections such as urinary tract infections, pneumonia and bacteremia (Aloush et al.) [15]. While the high level of multidrug resistant *Staphylococcus aureus* constituted a treat to the consumer's health. None of the *Salmonella* species was susceptible to chloramphenicol and cephalexin. Majority of the isolates were highly susceptible to ofloxacin, ciprofloxacin and gentamicin. This high level of susceptibility is evidence that these drugs are the best in the treatment of gastroenteritis in Elele community and this could be attributed to the less prescription of those drugs by physicians as well as high cost of the drugs which probably restricted its procurement and misuse thereby reducing the emergence

Table 4a Antibiotic susceptibility patterns of Gram negative bacteria isolated from fresh vegetable leaves sold in Elele market, Rivers State, Nigeria (%)

Isolate	Total no. of isolate	PN (25 µg)	C (30 µg)	CN (10 µg)	COX (15 µg)	T (25 µg)	CPX (10 µg)	SXT (25 µg)	OFX (10 µg)	CRO (30 µg)	AU (10 µg)
E. coli	128	57(44.5)	37(28.9)	101(78.9)	110(98.9)	29(22.7)	122(95.3)	34(26.6)	128(100.0)	98(76.6)	67(52.3)
E. aerogenes	80	32(40.0)	34(42.5)	73(91.3)	68(85.0)	22(27.5)	80(100.0)	37(42.3)	80(100.0)	43(53.8)	41(51.2)
Citrobacter species	13	7(53.8)	5(38.5)	11(84.6)	6(46.1)	5(38.5)	13(100.0)	8(61.5)	13(100.0)	11(84.6)	9(69.2)
A. *A. faecalis*	20	11(55.0)	9(45.0)	18(90.0)	12(60.0)	10(50.0)	19(95.0)	7(35.0)	20(100.0)	14(90.0)	13(65.0)
P. aeruginosa	36	3(8.3)	1(2.8)	10(27.8)	2(5.6)	1(2.8)	20(55.6)	1(2.8)	21(58.3)	4(11.1)	1(2.8)
Salmonella species	9	1(11.1)	0(0.0)	6(66.7)	2(33.3)	0(0.0)	9(100.0)	1(11.1)	9(100)	5(55.6)	1(11.1)
Shigella species	24	5(20.8)	0(0.0)	21(89.5)	3(12.5)	2(8.3)	24(100.0)	4(16.7)	22(91.7)	20(83.3)	2(8.3)

E. coli: *Escherichia coli*; PN: Ampicillin; CPX: Ciprofloxacin; *E. aerogenes*: *Enterobacter aerogenes* C: Chloramphenicol; SXT: Cotrimoxazole; A: Alcaligenes; CN: Gentamicin; OFX: Ofloxacin; P: Pseudomonas COX: Cephalexin; CRO: Ceftriazone; T: Tetracycline; AU: Augnentin

Table 4b Antibiotic susceptibility patterns of Gram positive bacteria isolated from fresh vegetable leaves sold in Elele market, Rivers State, Nigeria (%).

Isolate	TN of isolate	APX (30 µg)	E (30 µg)	PEF (10 µg)	AM (30 µg)	CN (10 µg)	SXT (25 µg)	CPX (10 µg)	S (10 µg)	Z (20 µg)	AU (10 µg)
Bacillus cereus	11	3(27.3)	37(28.9)	1(9.1)	2(18.2)	9(81.9)	5(45.5)	9(81.8)	8(72.7)	7(63.6)	4(36.4)
Micrococcus species	16	6(37.5)	34(42.5)	7(43.8)	4(25.0)	15(93.8)	8(50.0)	14(87.5)	10(62.5)	11(68.8)	6(37.5)
Staphylococcus aureus	100	66(66.0)	5(38.5)	47(47.0)	37(37.0)	97(97.0)	20(100.0)	100(100.0)	51(51.0)	85(85.0)	70(70.0)

TN: Total number; SXT: Cotrimoxazol; APX: Ampiclox; CPX: Ciprofloxacin; E: Erythromycin; S: Streptomycin; PEF: Pefloxacin; Z: Zinnat; AM: moxicillin; AU: Augmentin; CN: Gentamicin

of most bacterial isolates to these drugs. Ciprofloxacin and oflaxacin (Quinolones) have the ability to inhibit DNA gyrase in the bacterial cells and so, lead to lysis of the bacterial cells (BNF) [16]. Most bacteria also demonstrated resistance to ampicillin, chloramphenicol, tetracycline, cotrimazole and augmentin. This is an evidence of earlier exposure of the isolates to these drugs which may enhance the development of resistance [17].

The results of this present study indicate the poor hygienic condition of fresh vegetables sold in Elele community and the consumers are at risk of contacting food borne infections; also the level of multidrug resistant strains is of public Health concern [18,19]. Though the vegetables are properly de-contaminated after sales at the point of preparation/cooking, it is also very necessary for the de-contamination to be done before sales. There is need to educate the vendors and consumers on good sanitary practices during processing, display and sale of vegetables and also dangers associated with misuse of antibiotics.

Conflict of Interest

The authors declare that we have no conflict of interest.

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