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Microbial and Parasitic Contamination on Vegetables Collected From Retailers in Main Market, Akure, Nigeria

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Abstract Laboratory investigations were carried out on six different vegetable samples; *Amaranthus cruentus* (Amaranth), *Talinum triangulare* (Waterleaf), *Solanecio biafrae* (Worowo), *Brassica olerecea* (Cabbage), *Lactuca sativa* (Lettuce) and *Daucus carota* (Carrot) purchased from retailers in main market, Akure, Nigeria to determine the microbial and parasitic contamination. One part of each sample was washed with distilled water while the other part was washed with physiological saline. For parasitological analysis, the solutions were centrifuged, decanted and viewed under the microscope while culturing was done for microbial analysis using the pour plate technique. Only *L. sativa* was contaminated with helminth*; Ascaris lumbricoides*. All samples were found to be populated with various species of microorganisms. Seven bacteria belonging to different genera and six fungi were isolated randomly and identified from the vegetables. Bacteria include *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typii* and *Proteus vulgaris* while fungi are *Aspergillus niger*, *Saccharomyces* sp, *Penicillium* sp, *Rhizopus stolonifer*, *Fusarium* sp and *Mucor mucedo*. The total bacterial count ranged from 3.2×10^6 to 7.2×10^6cfu/g for samples washed with distilled water and 1.6×10^6 to 4.8×10^6cfu/g for samples washed with physiological saline. *S. aureus* had the highest occurrence of 25% while *Salmonella typhii* had the least occurrence of 10%. The fungal count ranged from 3.0×10^5 to 5.0×10^5sfu/g for samples washed with distilled water and 1.0×10^5 to 3.0×10^5sfu/g for samples washed with physiological saline. *R. stolonifer* had the highest percentage of occurrence of 33.3%. *Saccharomyces* had the occurrence of 22.2% and *Penicillium* sp, *A. niger*, *Mucor mucedo*, *Fusarium* sp had the least occurrence of 11.1%. This study showed the presence of organisms of health significance on retail vegetables. Reduction of risk of human illness associated with raw produce can be achieved through controlling points of potential contamination.

Keywords: *vegetables, bacteria, helminth, physiological saline, distilled water*

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1. Introduction

Consumption of leafy vegetables has increased in recent years because of their nutritional value and beneficial health effects [1]. Vegetables harbour a wide range of microbial contaminants which undermine their nutritional and health benefit thus increasing outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables [2]. The number of outbreaks of food borne illnesses associated with consumption of fresh vegetables has increased due to the increase in demand for fresh produce [3]. Surveillance of vegetables has indicated that these vegetables can be contaminated with various bacterial pathogens, including *Salmonella* sp, *Shigella* sp, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* at any of several points from the field through to the time of consumption [4]. Several factors contributed to increase in occurrence and outbreaks of numerous food borne diseases associated with raw fruits and vegetables. Some of the factors are globalization of food supply, introduction of pathogens into new geographical areas through import, use of untreated waste water and water supplies contaminated with sewage, manure as fertilizers for crop production, irrigation and various agronomic practices, post-harvest handling, and hygienic conditions of preparation in food service or home settings [5]. High incidence of human intestinal parasites has been found in communities that consume raw vegetables, especially where the vegetables are cultivated on farm lands fertilized with untreated human and animal sewage [6]. Although most processors and consumers assume that washing fresh vegetables and fruits will reduce the microbial load on their surfaces, but studies have shown that water washing alone is not effective in reducing microbial population on the fresh vegetables [4]. This research is therefore aimed at determining the carriage rate and identification of human

intestinal helminth ova and associated microorganisms on some selected leafy vegetables.

2. Materials & Methods

2.1. Sample Collection

A total of six (6) types of fresh vegetables (*Daucus carota, Amaranthus cruentus, Lactuca sativa, Brassica oleracea, Solanecio biafrae* and *Talinum triangulare*) were collected from Akure main market using a random sampling technique. Mature leaves of the selected vegetables were sampled at early maturity according to methods used by [7,8]. All samples were collected aseptically in a sterilized universal container and plastic bags and transported to The Federal University of Technology, Akure for laboratory processing [9]. Analysis was conducted within 24 hour of arrival at the Microbiology Laboratory of the University.

2.2. Parasitological analyses of vegetables

Different solvents were assayed for the washing of vegetable samples; these include sterile water and physiological saline solution (0.90% NaCl). In the laboratory, 100g of each fresh vegetable sample was chopped into small pieces and put into a clean beaker containing 500ml physiological saline solution (0.90% NaCl), to wash the sample. After removing fragments of the vegetable sample from the washing saline using clean forceps, it was kept for 24 hour to allow sedimentation to take place. The same experiment was carried out with the use of 500ml sterile water for each of the samples but was washed two times. After 24 hour sedimentation, the top layer of the washing solvent was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 revolution/minute for 20 minutes by HERMLE Z200A centrifuge.

After discarding the supernatant, the residue was mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope for the presence of intestinal helminth parasites or their ova, cysts of *E. histolytica* and *G. intestinalis* in the vegetable samples [10,11]. These were identified as described by [12].

2.3. Microbiological Analysis of Vegetables

Vegetable samples were washed with 100ml of saline water and 100ml of distilled water respectively into different sterile containers. The microbiological assay of sampled vegetables was determined by the pour plate technique as described by [8]. 1ml of dilution 10^{-4} and 10^{-5} were assayed using nutrient, MacConkey and potato dextrose agar as growth media. The plates were observed for growth after 24 hours and 72 hours respectively and the numbers of colonies on the plates were counted [13].

2.4. Statistical analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance (ANOVA) and means were compared by duncan multiple range test (DMRT) using SPSS 18.0 version. Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Helminth Parasite Ova and Microbial Load of Vegetable Samples

Ascaris lumbricoides ova were found in the distilled water solution and physiological saline solution of lettuce. Other samples were not contaminated with human intestinal helminth parasites as shown in Table 1. The total bacteria count on nutrient agar ranged from 3.2×10^6 to 7.2×10^6 cfu/g. In all the samples washed with distill water, *D. carota* was found to have the highest microbial load followed by *B. oleracea, T. triangulare, L. sativa, A. cruentus* and *S. biafrae* was the least contaminated on nutrient agar. The microbial count on MacConkey agar ranged between 1.0×10^6 to 5.2×10^6 cfu/g. Table 2 shows the highest microbial count on MacConkey agar to be found in *D. carota*, followed by *B. oleracea, S. biafrae, T. triangulare, L. sativa* and the least count was found in *A. cruentus.* The fungal count for distilled water ranged from 3.0×10^5 to 5.0×10^5sfu/g. The highest fungal count was found in *D. carota* and *L. sativa* followed by *A. cruentus* and the least fungal count was found in *B. oleracea, S. biafrae* and *T. triangulare* as shown in Table 2. The total bacteria count on nutrient agar ranged from 1.6×10^6 to 4.8×10^6 cfu/g. Of all the samples, *B. oleracea* was found to have the highest microbial load followed by *L. sativa, D. carota, S. biafrae, A. cruentus* and *T. triangulare* was the least contaminated. The bacteria count on MacConkey agar ranged from 5.0×10^5 to 3.5×10^6. *D. carota* has the highest microbial count on MacConkey agar, followed by *L. sativa, S. biafrae, B. oleracea* and the least count were found in *A. cruentus* and *T. triangulare*. The fungal count for physiological saline was between 1.0×10^5 to 3.0×10^5 (Table 3). The highest fungal count was found in *D. carota* and *L. sativa* followed by *A. cruentus, B. oleracea* and *T. triangulare* while the least count was found in *S. biafrae* as shown in Table 3.

Table 1. Ova of Intestinal Helminth Parasite Encountered In Vegetable Samples

Samples	Distilled Water	Normal Saline
Amaranthus cruentus	No cyst or ova	No cyst or ova
Talinum triangulare	No cyst or ova	No cyst or ova
Solanecio biafrae	No cyst or ova	No cyst or ova
Brassica oleracea	No cyst or ova	No cyst or ova
Lactuca sativa	*Ascaris lumbricoides*	*Ascaris lumbricoides*
Daucus carota	No cyst or ova	No cyst or ova

3.2. Identification and Distribution of Isolated Organisms

The seven bacteria and six fungi species isolated and identified from vegetable samples are Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Salmonella typii, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli., Penicillium sp, Aspergillus niger, Mucor mucedo, Rhizopus stolonifer, Fusarium sp and Saccharomyces sp were organism isolated and identified as contaminant of vegetable samplesas shown in Table 4.

S. aureus have 25% occurrence rate, P. vulgaris, E. coli and B. subtilis have 15% occurrence rate and P. aeruginosa, S. marcescence and S. typii have the least occurrence rate of 10%. Among the fungi encountered, R. stolonifer have the highest percentage of occurrence of 33.3% followed by Saccharomyces sp. with 22.2% and Penicillium sp, Aspergillus sp, Mucormucedoand Fusariumsp having the lowest occurrence of 11.1% as shown in Table 5.

Table 2. Mean Microbial Load of Vegetable Samples Washed With Distilled Water

Samples	Total Bacteria Count On Nutrient Agar (cfu/g)	Total Bacteria Count On MacConkey Agar (cfu/g)	Total Fungi Count On Potatoes Dextrose Agar (sfu/g)
Amaranthus cruentus	$5.6\times10^{6}\pm0.02$	$1.0\times10^{6}\pm0.01$	$4.0\times10^{5}\pm0.12$
Talinu mtriangulare	$6.4\times10^{6}\pm0.02$	$2.1\times10^{6}\pm0.07$	$3.0\times10^{5}\pm0.35$
Solaneciobiafrae	$3.2\times10^{6}\pm0.11$	$2.5\times10^{6}\pm0.08$	$3.0\times10^{5}\pm0.32$
Brassica oleracea	$6.5\times10^{6}\pm0.56$	$2.6\times10^{6}\pm0.24$	$3.0\times10^{5}\pm0.90$
Lactuca sativa	$5.7\times10^{6}\pm0.32$	$2.0\times10^{6}\pm0.03$	$5.0\times10^{5}\pm0.32$
Daucuscarota	$7.2\times10^{6}\pm0.05$	$5.2\times10^{6}\pm0.60$	$5.0\times10^{5}\pm0.31$

Table 3. Mean Microbial Load of Vegetable Samples Washed With Physiological Saline

Samples	Total Bacteria Count On Nutrient Agar (cfu/g)	Total Bacteria Count On MacConkey Agar (cfu/g)	Total Fungi Count On Potatoes Dextrose Agar (sfu/g)
Amaranthus cruentus	$2.4\times10^{6}\pm0.01$	$5.0\times10^{5}\pm0.15$	$2.0\times10^{5}\pm0.56$
Talinum triangulare	$1.6\times10^{6}\pm0.04$	$5.0\times10^{5}\pm0.26$	$2.0\times10^{5}\pm0.32$
Solanecio biafrae	$2.6\times10^{6}\pm0.35$	$1.1\times10^{6}\pm0.91$	$1.0\times10^{5}\pm0.80$
Brassica oleracea	$4.8\times10^{6}\pm0.27$	$1.0\times10^{6}\pm0.15$	$2.0\times10^{5}\pm0.35$
Lactuca sativa	$3.9\times10^{6}\pm0.08$	$1.2\times10^{6}\pm0.64$	$3.0\times10^{5}\pm0.44$
Daucus carota	$3.2\times10^{6}\pm0.31$	$3.5\times10^{6}\pm0.36$	$3.0\times10^{5}\pm0.61$

Table 4. Morphological and Biochemical Characteristics of Bacteria Isolates

Organism	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Serratiamarcescens*	*Salmonella typhii*
Cultural Characteristic							
Color	White	Creamy	Dirty white	White	Green	Red	White
Shape	Circular	Cicular	Spread	Irregular	Circular	Circular	Circular
Edge	Entire	Entire	Fimbranated	Rhizoid	Entire	Entire	Lobate
Elevation	Raised	Convex	Flat	Flat	Flat	Low convex	Raised
Surface	Smooth	Dull	Dull	Rough	Smooth	Smooth	Dull
Grams reaction	+ Cocci	- Rod	- Rod	+ Rod	- Rod	- Rod	- Rod
Biochemical Test							
Motility	-	+	+	+	+	+	+
Spore	-	-	-	+	-	_	-
Catalase	+	+	+	+	+	+	+
Coagulase	+	-	-	-	-	-	-
Citrate Utilization	-	-	W	+	+	+	-
Urease	--	-	+	W	+	-	-
Oxidase	-	-	-	-	+	-	-
Indole	-	+	-	+	-	-	-
Starch hydrolysis	+	-	+	-	-	-	-
Sugar utilization							
Glucose	A/G	A/G	A/G	A	A	A/G	L
Lactose	A	A/G	-	A	-	-	-
Sucrose	A/G	L	A	L	-	A/G	-
Maltose	A	A	A	A/G	-	A	A
Mannitol	A	A/G	-	A/G	A	A	A/G
Arablnose	A	A	-	A/G	L	-	-
Inositol	A	-	-	A	-	L	-

Table 5. Morphological characteristics of isolated fungi

Morphological Characteristics Of Isolates	**Isolates**
White cottony with small black spot. Sporangia have well developed collumella which is hemispherical in shape. Sporangiophores arises from the nodes where the rhizoids form.	*Rhizopus stolonifer*
Creamish in color and oval in shape (spore). The collula is smooth and very small. It has a branched cell.	*Saccharomyces* sp.
White cottony mycelium. Conidiophores variable slender, stout short and branch irregular. Macroconidia are fusiform, slightly curved, pointed at the top and mostly in three septate. Microconidia are abundant but not in chain.	*Fusarium* sp
White base spread dotted black spores. Spores are black, smooth, regular and bore within the sporangium. The collumella are round. Sporangiosphores are transparent, ellipsoidal and smooth walled.	*Mucor* sp
Black spores on the media. It produced septate, branching mycelia. Conidiophores are upright, radiating from the entire surface, turning dark towards vesicle. Conidia heads are large, globose becoming radiate and biserrate.	*Aspergillus niger*
Light blue. It possesses septate mycelium bearing single conidiophores which are branched near the apex and ends in phialides that carried conidia.	*Penicillium* sp

4. Discussion

This study revealed the carriage rate of human intestinal helminth ova and associated microorganisms in some selected leafy vegetables. In line with [10], the presence of only the ova of *A. lumbricoides* was found in lettuce in this study whereas the presence of helminths and other parasites on vegetables were observed in the study of [9,14]. This present study did not observe any human intestinal helminths, their ova any other parasite in other vegetable samples. This is not unexpected as this could be due to prewashing of the samples in clean water by farmers or retailers before sales and high level of hygiene could have been maintained during pre-harvest or post-harvest handling.

The observed difference in prevalence rates of the different intestinal helminth parasite ova from fresh vegetables was also reported by [14]. Several factors may contribute to such differences; geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and post-harvest handling methods of such vegetables.

A. lumbricoides is the causative agent of ascariosis. The reservoir host of the parasite is man. The eggs contaminates the soil or vegetable though promiscuous defecation or through the use of manure as organic fertilizer on farmland. The eggs are ingested by humans with contaminated food, soil and less frequently drinking water [15]. The occurrence of *A. lumbricoides* in some stream, river and other sources of water has been reported by the work of [11,16] which is also a predisposing factor to the contamination of vegetables when such water is used for irrigation.

The high microbial load obtained from the isolation of microorganism from the vegetable samples could probably be directly linked to the recorded use of waste water in irrigation for watering the field, or the manure used for fertilization and the unhygienic condition of the area where the vegetables were being grown and sold. The result correspond to the findings of [17] who reported that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of feaces or untreated sewage and pathogens existing in the soil or water can be the source of both pre- and post-harvest contamination respectively. The slight variation in the microbial load from other sources can be traced to prewashing of vegetables with portable water.

Among the organisms encountered during this study *Staphylococcus aureus* with the highest occurrence is in agreement with the studies of [7]. Poorna [18] also showed presence of *Salmonella, Serratia*, *Enterobacter*, *Staphylococcus aureus*, *E. coli* and *P. aeruginosa* in vegetables and fruits. *S. aureus* is a normal microbial flora of the mucus membrane and on the human skin. It is an opportunistic pathogen and enterotoxigenic strains which are known to cause serious food borne disease and has been reported that ingestion of the thermostable enterotoxins, rather than the bacterium itself is responsible for food borne illness [7]. They cause boils, abscesses, post-operative infections, toxic shock syndrome and food poisoning in man [13].

Other organisms encountered during the study and their respective health implications include *Bacillus subtilis* causing diarrhoeal. The occurrence of *Bacillus subtilis*in vegetables agrees with the findings of [19] who reported that estimated 27,000 cases of food borne illness are due to *Bacillus species*. *Bacillus spp*. are part of the natural flora and normally lives in the soil. They are among the most common vegetable spoilage bacteria [13], though some *Bacillus* species are capable of causing food borne illness.

Table 6. Microorganisms isolated from Vegetables

Sample	Microbial Isolates
Amaranthus cruentus	*Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Escherichia coli, Rhizopus stolonifer, Saccharomyces* sp
Talinum triangulare	*Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marscescens, Escherichia coli, Aspergillus niger, Saccharomyces* sp
Solaneciobiafrae	*Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Fusarium* sp
Brassica oleracea	*Salmonella typhii, Escherichia coli, Staphylococcus aureus, Rhizopus stolonifer*
Lactuca sativa	*Proteus vulgaris, Salmonella typhii, Serratia marscescens, Penicillium* sp
Daucuscarota	*Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Mucor mucedo, Rhizopus stolonifer*

The occurrence of *E. coli* may be linked to animal dung and manure used during the cultivation of vegetables as fertilizers. Other organisms encountered include *S. typhii* which has been reported to be responsible for typhoid fever [20] and several cases of an outbreak of typhoid fever have been associated with consumption of contaminated vegetable grown in or fertilized with contaminated soil or sewage [21].

Pseudomonas aeruginosa is a prominent inhabitant of soil and organism is responsible for diseases of vegetables like angular leaf spot of many vegetables it has become an important cause of infection and It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia [22]. It invades burns area, causes septic shock and responsible for cystic fibrosis in human [23]. They are also associated with spoilage of vegetable.

Michael *et al., [13]* described *Proteus vulgaris* as an organism thatoccur in the intestine of human and in a wide variety of animals, in polluted water, soil and they are opportunistic pathogens. Michael *et al.*, [13] also reported *Serratia* sp. that they were once thought to be harmless and it is now clear that they can be opportunistic human pathogen and are particularly prone to infect hospitalized patients.

The fungi isolate *Rhizopus stolonifer* and *Mucor mucedo* has been reported to cause food spoilage. They grow on fruits and vegetables [13]. These fungi grow on the surface of moist, carbohydrate-rich foods, such as breads, fruits, and vegetables [23]. The presence of *Penicillium* sp and *Aspergillus niger* agreed with the study of Akinyele *et al.*, [7] and could be due to the fact that these organisms are spore formers and are known as common environmental contaminants [13,23] nevertheless, they have been implicated as food borne pathogens [7].

Table 7. Distribution Of Bacteria And Fungi Contaminant In Vegetable

ORGANISM	*Amaranthus cruentus*	*Talinum triangulare*	*Solanecio biafrae*	*Brassica oleracea*	*Lactuca sativa*	*Daucus carota*	Number of Isolates	% of Occurrence
Bacteria					sw			
S. aureus	+	+	+	+	-	+	5	25
P. vulgaris	+	-	+	-	+	-	3	15
E. coli	+	+	-	+	-	-	3	15
B. subtilis	+	-	+	-	-	+	3	15
P. aeruginosa	-	+	-	-	-	+	2	10
S. marcescence	-	+	-	-	-	+	2	10
S. typhii	-	-	-	+	+	-	2	10
TOTAL	**4**	**4**	**3**	**3**	**2**	**4**	**20**	**100**
FUNGI								
Penicilliumspp	-	-	-	-	+	-	1	11.1
Aspegillusspp	-	+	-	-	-	-	1	11.1
Mucormucedo	-	-	-	-	-	+	1	11.1
R. stolonifer	+	-	-	+	-	+	3	33.3
Fusariumsp	-	-	+	-	-	-	1	11.1
Saccharomyces. Spp	+	+	-	-	-	-	2	22.2
TOTAL	**2**	**2**	**1**	**1**	**1**	**2**	**9**	**100**

Several fungi from different region has been isolated and identified to be associated with fruits and vegetables. The most common fungi found in a study by Akintobi *et al*., [24] were *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and yeasts. Baiyewu *et al*., [25] had also reported that *Rhizopus nigricans*, *Aspergillus flavus*, *Aspergillusniger* and *Fusarium moniliforme* among others, were responsible for post-harvest losses in pawpaw in South Western, Nigeria. The isolation of *Rhizopus stolonifer* from vegetables further confirmed the studies of Efiuvwerwere, [26], Chuku *et al*., [27], Akinmusire [28], and Akintobi *et al*., [24] who reported that *Fusarium* sp, and *Rhizopus stolonifer* were responsible for the soft rot of tomato. Onyia *et al*. [29] also reported that *Fusarium monilifome*, *Aspergillus niger* and *Rhizopus.*

In this study, the high prevalence of fungi, bacteria and parasites was further enhanced by unhygienic mode of transportation of these consumable products. Also local practice of using organic manure, such as human, animal and poultry dropping as fertilizer contributed immensely to the occurrence of the pathogen research. The contamination of vegetables by pathogenic bacteria, fungi and parasites could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation [26].

5. Conclusion and Recommendation

This is a preliminary study of an on-going research, it showed the presence of organisms that have serious public health significance while others fasten spoilage of the vegetables. High numbers of these microorganisms in raw consumed vegetables and salad produce would lead to the consumer's illness with symptoms of the particular or combined microbial presence. Reduction of risk for human illness associated with raw product can be better achieved through controlling points of potential contamination in the field during harvesting, during processing or shipment, storage or distribution in the retail markets, food services facilities or home. The result of this study also shows that salt washing can reduce microbial loads on vegetables by lysing the cell wall.

It is therefore necessary and important for regulatory authorities in conjunction with the government to formulate a technique or safe way of producing, handling, processing, storing and retailing leafy vegetables especially in developing countries such as Nigeria. To control food borne infections during post-harvest handling, vegetables should be thoroughly washed with clean portable water before consuming raw and before cooking, addition of salt to the rinse water may also reduce microbial load as indicated in this study. It is therefore necessary and important that both the farmer who harvests the vegetables for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables.

Seminars, random examinations and research can also be organized and carried out on sellers and vegetables to educate sellers on the implication and hazard of improper storage, handling and display of leafy vegetables.

Further studies on the enteroxigenicity tests for *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* can be conducted also the effect of different salt concentrations on microorganisms isolated from leafy vegetables.

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