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Microbiological and Parasitological Assessment of Vegetables Sold At Owena Ijesa Market Osun State, Nigeria

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Abstract: Vegetable can serve as a source of food and it can be contaminated by microorganisms and parasite which can serve as a source of diseases. Laboratory investigations were carried out on four different vegetable samples: *Cochorus olitorius* (Ewedu), *Celosia argentea* (Shoko), *Daucus carota* (Carrot), *Brassica olerecea* (Cabbage), purchased from retailers in Owena-Ijesa, Nigeria. Standard Microbiological and parasitological analysis were carried out. *Staphylococcus* sp., *Bacillus* sp., *Proteus* sp., *Enterobacter* sp., *Salmonella* sp., *Enterococcus* sp., *Vibrio* sp., *Paenibacillus* sp., *Pseudomonas* sp., *Escherichia* sp., *Brevibacillus* sp. and seven fungi *Saccharomyces* sp., *Penicillium* sp, *Aspergillus niger, Rhizopus stolonifer, Trichoderma harizianum, Mucor* sp. and *Fusarium* sp. were isolated and identified from the vegetables. The highest total bacterial count was 8.0×10^4 and lowest fungal count was 1×10^3 in cabbage. *E. coli* had the highest occurrence of 18.18% and was found on all marketed vegetable samples used in this study while *P. aeroginosa*, *B. subtilis* and *B. brevis* had the least occurrence of 3.03%. *Saccharomyces* sp, *Penicillium* sp, and *Aspergillus niger* had the highest occurrence of 20% while *Rhizopus stolonifer*, *Trichoderma harizianum*, *Mucor* sp. and *Fusarium* sp. had the least occurrence of 10%. This study showed the presence of organisms of health significance on retail vegetables, reduction of risk of human illness can be achieved through controlling points of potential contamination from handling, transportation, processing of raw vegetables and strict government laws banning the use of untreated fertilizer on farm produce.
Keywords: Vegetables, Microbiological and parasitological analysis, Contamination, Diseases.

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Introduction

Vegetables consumption has increased in Africa over the past decade and it represent valuable sources of nutrients rich in essential components important to man can which is attributed to the exposure of the health benefits of consuming these vegetables (Heaton and Jones, 2007). There are studies that show that consumption of vegetables help prevent diseases and cancer (Kwabena *et al.*, 2018). Vegetable consumption is responsible for providing protein, vitamins, mineral, fibres and other nutrients at rural environments which

are not normally present in the daily diets (Mohammed and Sharif, 2011). While the importance and impact of these vegetables cannot be disputed, they still represent a major risk to the health of the consumers. Diseases transmitted through consumption of food and drinks is still globally prominent and vegetables, especially vegetables consumed raw and not properly washed are part of the major contributors to this trend (Ajayi *et al* 2016).

Vegetables can act as vehicles for the transmission of parasitic and microbial infection when contaminated (Beauchat, 2002). The evidence of intestinal parasites on vegetables most likely denotes the planting and harvesting practices employed, post-harvesting techniques and even storage and preservation practices. Some enteropathogens like *Salmonella* and *Escherichia coli* O157:H7 normally present in vegetables such as lettuce, spinach and tomatoes (Lihua *et al*, 2008).

Epidemiological studies have revealed that in the Southwestern part of Nigeria, the practice of using untreated wastewater for irrigation of farm produce and consumption of such water irrigated vegetables when unwashed and not properly cooked may lead to severe parasitic infections.

The safety of these vegetables in Nigeria raises serious concerns because there are no rules or laws that govern and enforce strict sanitation conditions and practices in harvesting, processing, handling and packaging of these vegetables. Taking into heart the conditions of these vegetables, how they are handled and sold, it comes as no surprise that the safety of these vegetables could cause deleterious effects and thus not meeting health standard. Hence this study aims to detect the existence of eggs, cysts and oocysts of parasites present in marketed vegetables sold at Owena-Ijesa market in Osun state. It also aims to investigate the microbial contamination present from marketed vegetables.

Materials and Methods

Study Area

The study was carried out at Owena-Ijesa market located at Owena, Osun state Nigeria. Four vegetables *Cochorus olitorius* (Ewedu), Lagos spinach (Shoko) *Celosia argentea*, *Daucus carota* (Carrot) and *Brassica aleracea* (Cabbage) popularly consumed among the villages were bought unwashed from the market. Thirty (30) of each of the vegetables were randomly picked from the vendors for a period of one month. They were aseptically collected in polythene bags due to the purpose of study. The bags were labelled and transferred immediately to the microbiological laboratory at Joseph Ayo Babalola University, Ikeji-Arakeji.

Laboratory procedures

Parasitological analysis of vegetables

100g of the vegetable sample was weighed and washed separately in beakers containing 250ml of distilled water and normal saline (0.90% NaCl) each for detaching the parasitic stages (ova, larva, cysts, and oocysts) commonly assumed to be associated with vegetable contamination. Samples were washed vigorously by shaking and vegetables were removed and discarded into waste bins.

Each of the beakers were left overnight for sedimentation to take place. The supernatant was discarded leaving about 15ml at the bottom. 10ml of the washed sample was sieved using fine guaze into 10ml centrifuge tube and centrifuged at 3000rpm for five minutes using a 800D electric low speed centrifuge (Dada *et al*., 2015). Supernatant was decanted and sediment

were stained with lugol's iodine and examined under light microscope under 10x and 40x objective lens respectively. Intestinal parasites were identified using techniques described by (Abougrain *et al*., 2010).

Microbial analysis

1ml of the sample was serially diluted and plated into petri-dishes for bacterial and fungal analysis. 1ml of the samples were drawn from 10^{-3} and 10^{-5} for fungi and bacteriarespectively and transferred to labelled plates after which homogenized mixture of nutrient agar, yeast extract agar and potato dextrose agar were poured and allowed to solidify.

Plates were inverted and incubated at 25°C for 72 hours for fungi and 37°C for 24 hours for bacteria. Plates were examined and pure culture was obtained. Colony counting was done and needed biochemical tests, microscopic and fermentative tests were done on each isolates (Chan *et al*., 2005).

Results

The study revealed that none of the 120 vegetables samples obtained from Owena-Ijesa Market within the one month of the study contained parasites. The fungal count ranged between 2.0×10^3 in Shoko to 2.0×10^5 in Cabbage (Table 2).

Bacterial count of freshly marketed vegetables ranged from 8.0×10^4 on Nutrient agar in Shoko and 8.0×10^4 on nutrient agar in Cabbage 1.5×10^4on Fungi such as *Saccharomyces* spp., *Penicillium* spp., *Aspergillus niger*, *Mucor* spp.,and *Fusarium* spp., were isolated from the samples. Bacteria isolated from the samples ranged from *Escherichia coli* on nutrient agar *Proteus mirabilis* (Table 2).

Escherichia coli had highest occurrence of 18.18% and was found on all marketed vegetable samples used in the study (Table 5). *Salmonella typhi* had the second highest occurrence and was found in three of the vegetable samples except *Cochorus olitorius* (ewedu).

Among the fungi isolated, *Saccharomyces* sp. *Penicillium* sp. and *Aspergillus niger* had equal number of occurrence of two in the vegetable samples (Table 6). *Saccharomyces* sp. and *Penicillium* sp. were present in *Cochorus olitorius* and *Celosia argentea* while *Aspergillus niger* was found in *Celosia argentea* and *Daucus carota*.

Table 1. Total bacteria count of freshly marketed vegetables

Samples	Nutrient agar (Cfu/ml)
EW 10^{-3}	8.0×10^1
EW 10^{-5}	2.0×10^4
SH 10^{-3}	4.5×10^2
SH 10^{-5}	2.0×10^4
CR 10^{-3}	3.0×10^2
CR 10^{-5}	1.0×10^4
CB 10^{-3}	3.4×10^1
CB 10^{-5}	1.5×10^4

Keyword: EW (Ewedu), SH (Shoko), CR (Carrot), CB (Cabbage), Cfu/ml (colony forming unit per ml)

Table 2. Total fungal count of freshly marketed vegetables

Samples	Potato dexterose agar (Cfu/ml)
EW 10^{-3}	2.0×10^{3}
EW 10^{-5}	4.0×10^{5}
SH 10^{-3}	3.0×10^{3}
SH 10^{-5}	8.0×10^{5}
CR 10^{-3}	3.0×10^{3}
CR 10^{-5}	2.0×10^{5}
CB 10^{-3}	1.0×10^{3}
CB 10^{-5}	2.0×10^{5}

Keyword: EW (Ewedu), SH (Shoko), CR (Carrot), CB (Cabbage), Cfu/ml (colony forming unit per ml

Table 3. Morphological Characteristics of fungi isolated from vegetables

Isolate ID	Cultural Characteristics	Microscopic Examination of Slide	Probable Organism
SH 10^{-3}	Shiny, flat, smooth creamy colonies	Single celled structures with branched cell	*Saccharomyces* sp.
EW 10^{-5}	Powdery olivaceous light yellow with a white margin	Septate mycelium bearing a single conidiophores which are branched near the apex and ends in phialides that carried conidia	*Penicillium*sp
SH 10^{-5}	White fluffy growth of colonies with elevated mycelium that turned black after 36 hours	Black with sulphur area on the surface single celled spore (conidia) in chains developing at the end of the sterigma arising from the terminal bud	*Aspergillus niger*
CR 10^{-3}	White conidia formed densely over the centre and in undiluting concentric rings	Conidia subglobose to ovoidal, Globose, intercalary hyphae and terminal phialides	*Trichoderma harizianum*
CR 10^{-5}	Whitish colony becoming with age	Non septate sporangiophore are directly opposite the branched rhizoids.	*Rhizopusstolonifer*
CB 10^{-3}	Smooth white base colony with dotted black spores	Ellipsodial smooth wall sporangiophores with round collumells	*Mucor* sp.
CB 10^{-5}	White cottony mycelium	Conidiophores are, slender branched irregularly	*Fusarium* sp.

Keyword: EW (Ewedu), SH (Shoko), CR (Carrot), CB (Cabbage),

Table 4. Morphological and Biochemical Characteristics of bacteria isolated from vegetables

Morphology						Biochemical Tests														
																Sugar Fermentation				
Isolate Id	Shape	Elevation	Edge	Optics	Pigmentation	Cell Shape	C.A	G.S	S.S	CO	C	CI	O	I	M	H_2S	G	S	L	F	Possible Isolate
EW	Irregular	Flat	Undulate	Transparent	White	Rod	Si	-	-	-	+	+	-	-	+	+	+g	-	-	-	*Proteus mirabilis*
EW CR	Irregular	Flat	Lobate	Transparent	White	Cocci	CS	+	-	+	+	+	-	-	-	-	+g	+g	+g	+g	*Staphylococcus aureus*
EW	Circular	Raised	Entire	Opaque	Cream	Cocci	CS	+	-	-	-	-	-	-	-	-	+g	+g	+g	+g	*Enterococcus faecalis*
SH SH CB	Irregular	Flat	Lobate	Translucent	Cream	Rod	CS	-	-	-	+	-	-	+	+	-	+g	+g	+g	-	*Escherichia coli*
SH	Circular	Flat	Entire	Translucent	Cream	Cocci	Si	-	-	+	+	+	+	+	+	-	+g	+g	+g	-	*Vibrio cholera*
SH CR	Circular	Convex	Entire	Translucent	White	Rod	CS	-	-	+	+	-	-	-	+	+	+g	-	-	+g	*Salmonella typhi*
SH CR	Circular	Flat	Undulate	Translucent	Cream	Rod	Si	-	-	+	+	+	-	-	+	-	+g	+g	+g	+g	*Enterobacter aerogenes*
CB	Irregular	Flat	Undulate	Transparent	Cream	Cocci	CS	+	-	-	+	-	-	-	-	+	+g	+g	+g	+g	*Staphylococcus epidermidis*
CB	Circular	Flat	Entire	Translucent	White	Rod	CS	+	+	-	+	-	-	-	+	-	+g	+g	-	+g	*Paenibacillus validus*

Keyword: C.A- Cell Arrangement; G.S- Gram Stain; S.S- Spore Staining; CO- Coagulase; C- Catalase; CI- Citrate; O- Oxidase; I- Indole; M- Motility; H_2S- Hydrogen Sulphide; G- Glucose; S- Sucrose; L- Lactose; F-Fructose; CS- Clusters; Si- Single; g- Gas; +- Positive; -- Negative

Table 5. Distribution of bacteria contaminant in vegetables

Organism	*Cochorus olitorius*	*Celosia argentea*	*Daucus carota*	*Bressica oleracea*	Number Of isolates	% of Occurrence
E. coli	+	+	+	+	6	18.18
B. subtilis	+	+	-	-	1	3.03
P. mirabilis	+	-	-	-	2	6.06
E. aerogenes	+	+	+	-	4	12.12
S. typhi	-	+	+	+	5	15.15
S. aureus	+	+	+	-	4	12.12
E. faecalis	+	+	+	-	2	6.06
V. cholera	-	+	+	-	3	9.09
P. validus	-	-	+	+	2	6.06
S. epidermis	-	-	+	+	2	6.06
P. aeroginosa	-	-	-	+	1	3.03
B. brevis	-	-	-	+	1	3.03
TOTAL	**6**	**7**	**5**	**6**	**33**	**100**

Keyword: + Present, - absent, *Cochorus olitorius* (ewedu), *Celosia argentea* (shoko), *Daucu carota* (Carrot), *Brasicca oleracea* (Cabbage).

Table 6. Distribution of fungal contaminant in vegetables

Organism	*Cochorus olitorius*	*Celosia argentea*	*Daucus carota*	*Bressica oleracea*	Number of isolates	% of Occurrence
Saccharomyces spp.	+	+	-	-	2	20
Penicillium spp.	+	+	-	-	2	20
Aspergillus niger	-	+	+	-	2	20
Rhizopus stolonifer	-	-	+	-	1	10
Trichoderma harizianum	-	-	+	-	1	10
Mucor spp.	-	-	-	+	1	10
Fusarium spp.	-	-	-	+	1	10
TOTAL	**2**	**3**	**3**	**2**	**10**	**100**

Keyword: + Present, - Absent, *Cochoruso litorius* (ewedu), *Celosia argentea* (shoko), *Daucuscarota* (Carrot), *Brasicca oleracea* (Cabbage).

Discussion

The research failed to establish parasitic infection of marketed vegetables brought and bought at owena market. This present study did not observe any human intestinal helminths, their ova, or any other parasite in the vegetable samples. It is possible that due to pre-washing of the samples in clean water by farmers or retailers before sales and the famers and food handlers maintained extreme level of hygiene at the time of harvesting or transportation (Dada *et al*., 2015).

The observed absence of any human intestinal helminths, their ova or any parasite in the fresh vegetable samples was also reported by (Kwabena *et al*., 2018) who observed only one parasite *Ascarislum bricoides* in association with lettuce. Various factors like the location of each village, quantity of samples, procedures of identification, quality of the water used for

planting and techniques of harvesting and preservation could be responsible for the differences. Twelve bacteria isolates were isolated from the vegetable samples. Seven fungi isolates were isolated from the vegetable samples. Each of the isolates were identified based on colonial, morphological, biochemical and fermentation tests.

The microbial load found on each of these vegetable samples could possibly be traced to the quality and type of water used during irrigation and also during processing and packaging. In addition, contact from humans and things in the environment could be responsible for the presence of some of these suspected parasites. *Escherichia coli* was found to be of highest occurrence which agrees with the studies of Akinyele *et al*. (2013). *E. coli* are a diverse group of bacteria found in food, water and intestine of people and animal. Although most strains are considered harmless, others have deleterious effects on health causing complications such as diarrhoea, respiratory illness and other illness (Ajayi *et al*., 2018).

Other bacterial organisms isolated from vegetable samples include *Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Enterobacter aerogens, Salmonella typhi, Enterococcus faecalis, Vibrio cholerae, Paenibacillus validus, Pseudomonas aeroginosa*. *S.aureus* is an opportunistic pathogen and its enterotoxigenic strains are popular for causing severe food borne illness (Dada *et al*., 2015). *V. cholera* is the causative agent of cholera. There are present in contaminated water and when ingested, causes diarrhea,vormitting and abdominal cramps. This study may have established a secondary source for the constant of occurrence of cholera among the villagers and inhabitants of the environs. The presence of *Bacillus subtilis* in vegetables was also reported by Mohammed and Sharif (2011), in their findings, it was established that *B. subtilis* is a causative agent of diarrhea and are soil dwelling microbes. They are capable of reducing the shelf life of vegetables and some are capable of causing diseases.

The presence of *S. typhi* could be as a result of farmers employing conventional means of fertilizing their plants with animal manure. Faeces are known to be contaminated with *S. typhi* which are causative agents of typhoid. *Pseudomonas aeroginosa* is a prominent cause of disease in plants causing angular leaf spot and has become very important to microbiologists. According to (Aloush *et al*., 2006), they cause spoilage of vegetables and are responsible for conditions such as pneumonia, cystic fibrosis and even urinary tract infections.

Fungi isolated from the vegetable samples include *Saccharomyces* sp., *Penicillium*sp, *Aspergillus niger, Rhizopus stolonifer, Trichoderma harizianum, Mucor* spp. and *Fusarium* spp. *Rhizopus stolonifer* and *Mucor* spp are popular causative agents of food spoilage, they are found of vegetables and sometimes fruits. The presence of *Penicillium* sp. and *Aspergillus niger* in this study is in agreement with the findings of Akinyele *et al*. (2013). These fungi isolates have been found to have potential food borne pathogenic implications.

The high occurrence of fungi and bacteria in this study is proof that the planting, harvesting, distribution, processing and handling techniques employed on these vegetables is one that raises a serious concern on the health implications it has on people. The use of conventional manure could also be responsible for the presence of these pathogens in the plants.

Conclusion

This study assessed the parasitic and microbial quality of four vegetable samples (Ewedu, Shoko, Carrot and Cabbage). This study revealed the absence of parasites in one hundred and

twenty samples of each of the four vegetables. The presence of high numbers of microorganisms in raw consumed vegetables and produce would lead to the consumer's illness with symptoms of the particular or combined microbial presence.

Recommendation

This study has revealed the implications of consumption of contaminated vegetables. Consumer's face a potential risk of ingesting one or more of these pathogens which could cause severe health damages. Due to this, the government of Nigeria should create laws that prohibit the use of untreated manure and water to be used for planting. Reduction of risk of human illness associated with raw product can be better achieved through controlling the point of potential contamination in the field during harvesting, processing, transporting, storage and distribution. Further studies on the effects of saline water on these microbial pathogens should also be carried out.

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