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Microorganisms of Public Health Significance Isolated from Salad Fruit and Vegetables in Dutsin-Ma, Katsina State, Nigeria

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ABSTRACT

Background and Objective: In developing nations, vendors mostly handle fresh salad fruits and vegetables under poor hygienic and sanitary conditions. The study investigated microorganisms of public health significance that contaminate common salad fruit and vegetables in Dutsin-Ma. **Materials and Methods:** The sampling was carried out between June and August, 2018. Samples of fruit [*Cucumis sativus* (cucumber)] and 3 vegetables [*Brassica oleracea* (cabbage), *Lactuca sativa* (lettuce) and *Allium cepa* (onion)] were each collected from 15 kiosks and randomly selected for the investigation. Microscopy was used for geohelminths, selective and non-selective media were used to isolate and enumerate the bacteria. All the species were identified based on morphology, biochemical tests (IMViC) and selective differential culture media. **Results:** *Ascaris lumbricoides* is the only geohelminths reported and only in *Lactuca sativa* (46.67%). *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* (bacteria) and *Fusarium* sp. and *Rhizopus stolonifer* (fungi) were randomly isolated and identified from the samples. The highest occurrence of 33.33% was of *S. aureus* while, the least occurrence of 8.33% was of each of *P. aeruginosa* and *P. vulgaris*. The fungal count showed *Rhizopus stolonifer* showed with the highest occurrence (75%) among the samples while the least (5%) occurred fungi was *Fusarium* sp. **Conclusion:** Salad fruit and vegetables in Dutsin-Ma are mostly contaminated with microorganisms of public health significance, with *Lactuca sativa* (lettuce) being the most contaminated with geohelminths, bacteria and fungi.

KEYWORDS

Fungi, bacteria, *Ascaris lumbricoides*, geohelminths, public health

INTRODUCTION

Microbial contamination has been established to be the main cause of foodborne illnesses, always linked with the intake of fresh fruits and vegetables[1,2]. Vegetables get contaminated with enteric bacteria, viral and parasitic pathogens during the process of cultivation, processing and even when serving[3]. The level of fruits and vegetable contamination is determined by several factors that include the use of contaminated water for irrigation, use of untreated or improperly composed manure for cultivation, faecal contamination from domestic animals and human beings, post-harvest handling by farmers,

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transportation, normal microflora of soil, transportation, unconscious handling by retailers and unhygienic conditions of preparation in food service or home settings[2,4,5]. Consumption of raw fruits and vegetables has been gaining attention as a very important means of both human and zoonotic pathogens[6]. Fresh fruits and vegetables are mostly consumed raw or lightly cooked to preserve the taste and nutrient contents, this serves as a potential source of various food-borne infections and disease outbreaks[7].

In Dutsin-Ma Town, inadequate supply of potable water for proper washing of these vegetables and poor handling by vendors, who mostly push water in trucks, contained in unclean kegs could be threats to public health. Some reports from the Northern Nigerian States have only reported on the contamination of fruits and vegetables with parasite cysts and ova (mostly geohelminths), with no exception to reports from Dutsin-Ma, Katsina State, where these vegetables are mostly cultivated by local farmers, packaged and transported to the markets or selling points in unhygienic condition[8]. Fresh salad fruits and vegetables in Dutsin-Ma are mostly handled by vendors under poor hygienic and sanitary conditions, mostly washed with water from contaminated sources. This study, therefore, investigated the contamination of fresh salad fruit and vegetables sold in Dutsin-Ma with microorganisms of public health significance.

MATERIALS AND METHODS

Study area: The study was conducted in Dutsin-Ma, Dutsin-Ma Local Government Area of Katsina State, Nigeria. Dutsin-Ma is a Town that lies on the latitude 12°26N and longitude 07°29E. According to Abaje *et al.*[9], Dutsin-Ma is surrounded by Kurfi and Charanchi LGAs to the North, Kankia LGA to the East, Safana and Dan-Musa LGA to the West and Matazu LGA to the Southeast. It has an area size of about 552.323 km^2 with a population of 169, 829 as of the 2006 National Census[10]. Recently, there has been a massive influx of people residents in Dutsin-Ma, who come as staff, students or visitors as a result of the presence of Federal University Dutsin-Ma, which was established in 2011 in the Local Government Area.

Collection of fruit and vegetable samples: Fruit and vegetable samples were collected from vendors at General Hospital Road, HayinGada (Abuja Road) and Isa Kaita-Low Cost Road, at fruits and vegetables, stands within Dutsin-Ma, between June and August, 2018. Vegetables [*Brassica oleracea* (cabbage), *Lactuca sativa* (lettuce) and *Allium cepa* (onion) and fruit, *Cucumis sativus* (cucumber)] which are among the most sold salad vegetables in the area constitute the research samples. In total, 120 samples were randomly collected from 15 vendors, at different locations for the investigation.

Laboratory analysis of samples

Parasitological analysis of fruit and vegetables: Sterile (distilled) water and physiological saline solution (0.90% NaCl) were used as solvents for the washing of fruit and vegetable samples. A total of 100 g of each fresh vegetable sample was chopped into small pieces, using a sterilized kitchen knife and put into a clean beaker containing 500 mL physiological saline solution (0.90% NaCl), to wash the sample. This was kept for 24 hrs to allow sedimentation to take place, after removing fragments of the samples from the washing saline using clean forceps. This was repeated using 500 mL of sterile water for each of the samples. Thereafter, after 24 hrs sedimentation, the top layer of the washing solvent was carefully discarded leaving 5 mL of the sediment. This was finally centrifuged at 2000 revolutions per minute for 20 min. The supernatant was discarded and the residue mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope for the presence of intestinal helminths or their eggs, cysts of *E. histolytica* and *G. intestinalis* in the vegetable samples as described by Su *et al.*[11] and Nwele *et al.*[12].

Microbiological analysis of fruit and vegetables: The microbiological assay of sampled fruits and vegetables was conducted by the pour plate technique as described by Dada and Olusola-Makinde[13]. A 1 mL of each dilution, 10^{-4} and 10^{-5} were assayed using Nutrient, MacConkey and Potato dextrose agar

as growth media. The bacteria were isolated and enumerated by growing them on selective and non-selective mediums such as nutrient agar used for Total Viable Bacterial Count (TVBC) and for Total Coliform (TC) and Faecal Coliform (FC) count MacConkey broth were used. One loop full culture from LB broth was streaked over selective media and kept for 24 hrs of incubation at an average temperature of 37°C and the numbers of colonies on the plates were counted. Contamination with microorganisms of public health significance was determined using the sedimentation concentration method, using normal saline and distilled water for washing, while the pour plate technique was adopted for culturing and microbial analysis.

Data analysis: Descriptive data of the findings are presented in Tables as Mean±Standard deviation. One-way Analysis of Variance (ANOVA) was used in testing the differences in the mean of contamination and data were further subjected to Duncan Multiple Range Test (DMRT) for mean separation using SPSS 18.0 version. Mean differences were considered significant at $p \leq 0.05$, 95% Confidence Interval (CI).

RESULTS

Results of the parasitic microscopy during the study revealed only *Ascaris lumbricoides* ova to contaminate *Lactuca sativa* (lettuce), the only vegetable in both the distilled water and physiological saline solution. The fruit and other two vegetable samples were not contaminated with human intestinal geohelminths in Table 1. Six different bacteria belonging to 6 genera and 2 fungi, of 2 genera were isolated randomly and identified from the fruit and vegetables. As presented in Table 2, *S. aureus* had the highest occurrence (33.33%) while, *Pseudomonas aeruginosa* and *Proteus vulgaris* had the least occurrence (8.33%). *Rhizopus stolonifer* had the highest occurrence (75%) and *Fusarium* spp., had the least occurrence (25%).

The total bacteria count on nutrient agar for samples rinsed with distilled water ranged from 4.3×10^6 to 6.2×10^6 CFU g^{-1}, with *Lactuca sativa* being the most contaminated with a bacterial load of $6.2\times10^6\pm0.32$ CFU g^{-1} while, the least bacterial load of $4.3\times10^6\pm0.08$ CFU g^{-1} was in *Cucumis sativus*. On MacConkey agar, the bacterial load was highest ($3.3\times10^6\pm0.49$ CFU g^{-1}) in *A. cepa* and least ($1.1\times10^6\pm0.91$ CFU g^{-1}) in *B. oleracea*. The highest (4.7×10^5 CFU g^{-1}) fungal count was found in *L. sativa* while the least (3.1×10^5 SFU g^{-1}) fungal count was in *A. cepa* in Table 3. The total bacterial count on nutrient agar for samples rinsed with physiological saline ranged from 1.0×10^6 to 5.1×10^6 CFU g^{-1}, with *Allium cepa* having the highest load, while, the least contamination was in *B. oleracea*. The bacteria count on MacConkey agar for physiological saline was lowest (1.0×10^6) in *A. cepa*

Table 1: Prevalence of intestinal helminth in fruit and vegetable samples collected in Dutsin-Ma

Samples	No. of samples examined	No. of positive samples	Positive (%)	Distilled water	Normal saline
Cucumis sativus	15	-	0	No cyst or ova	No cyst or ova
Brassica oleracea	15	-	0	No cyst or ova	No cyst or ova
Allium cepa	15	-	0	No cyst or ova	No cyst or ova
Lactuca sativa	15	7	46.67	*Ascaris lumbricoides*	*Ascaris lumbricoides*
Total	60	7	11.67	-	-

Table 2: Prevalence of bacteria contaminants in salad vegetables in Dutsin-Ma

Organism	*Cucumis sativus*	*Brassica oleracea*	*Lactuca sativa*	*Allium cepa*	Number of isolates	Occurrence (%)
Staphylococcus aureus	+	+	+	+	4	33.33
Proteus vulgaris	-	-	+	-	1	8.33
Escherichia coli	+	+	-	-	2	16.66
Bacillus subtilis	+	-	-	+	2	16.66
Pseudomonas aeruginosa	-	-	-	+	1	8.33
Salmonella typhi	-	+	+	-	2	16.66
Total	3	3	3	3	12	

Table 3: Mean microbial load of fruit and vegetable in Dutsin-Ma samples rinsed with distilled water

Samples	Total bacteria count on nutrient agar (CFU g^{-1})	Total bacteria count on MacConkey agar (CFU g^{-1})	Total fungi count on potatoes dextrose agar (SFU g^{-1})
Cucumis sativus	$4.3\times10^6\pm0.08$	$1.1\times10^6\pm0.91$	$3.2\times10^5\pm0.11$
Brassica oleracea	$5.4\times10^6\pm0.12$	$2.0\times10^6\pm1.71$	$3.3\times10^5\pm0.30$
Allium cepa	$5.5\times10^6\pm0.05$	$3.3\times10^6\pm0.49$	$3.1\times10^5\pm0.10$
Lactuca sativa	$6.2\times10^6\pm0.32$	$2.2\times10^6\pm0.41$	$4.7\times10^5\pm0.07$

Table 4: Mean microbial load of fruit and vegetable samples in Dutsin-Ma rinsed with physiological saline

Samples	Total bacteria count on nutrient agar (CFU g^{-1})	Total bacteria count on MacConkey agar (CFU g^{-1})	Total fungi count on potatoes dextrose agar (SFU g^{-1})
Cucumis sativus	$2.0\times10^6\pm0.13$	$4.2\times10^6\pm0.17$	$2.4\times10^5\pm0.21$
Brassica oleracea	$1.0\times10^6\pm0.06$	$4.4\times10^6\pm0.20$	$2.3\times10^5\pm0.18$
Allium cepa	$5.1\times10^6\pm0.30$	$1.0\times10^6\pm0.07$	$2.1\times10^5\pm0.27$
Lactuca sativa	$4.0\times10^6\pm0.17$	$1.2\times10^6\pm0.09$	$3.0\times10^5\pm0.22$

Table 5: Prevalence of fungi contaminant in vegetables

Organism	*Cucumis sativus*	*Brassica oleracea*	*Lactuca sativa*	*Allium cepa*	Number of isolates	Occurrence (%)
Rhizopus stolonifer	+	+	-	+	3	75
Fusarium spp.	+	-	-	-	1	25
Total	2	1	0	1	4	100

and highest (4.4×10^6) in *B. oleracea*. The fungal count for physiological saline was between 2.1×10^5 to 3.0×10^5 SFU g^{-1}, with the highest ($3.0\times10^5\pm0.22$) count in *L. sativa*, while, *A. cepa* had the least ($2.1\times10^5\pm0.27$) count in Table 4. *Rhizopus stolonifer* was present in all the vegetables, except in *Lactuca sativa*, with 75% occurrence while *Fusarium* spp., was present only in *Cucumis sativus*, with 25% occurrence in Table 5.

DISCUSSION

This study has established the contamination of salad fruit and vegetables with micro-organisms of public health significance. The contamination of the fruit and vegetable sampled might have resulted at any point between cultivation and handling by vendors. Several factors may be indicated that affect the variation of the microbial profile of vegetables including local microflora of soil cultivated, animal manure-derived flora, irrigation using sewage water, unhygienic transportation conditions and handling by the vendors[2]. The result corresponds to the findings of Buck *et al.*[14] and Biswas *et al.*[2], who reported that the practice of using untreated sewage, the presence of faeces in the environment and pathogens existing in the soil or water are sources of both pre and post-harvest contamination of these fruits. The high occurrence of *Staphylococcus aureus* reported in this study is similar to the report of Vishwanathan and Kaur[15], Akinyele *et al.*[16] and Biswas *et al.*[2], who reported high contamination of *Staphylococcus aureus* in fruits and vegetables. The high presence of *S. aureus* maybe because it is a common flora of man and vegetables can easily get contaminated with them when they come in contact with the human body. The common use of untreated animal dungs as organic manure during the cultivation of fruits and vegetables could be responsible for the occurrence of *E. coli* reported in this study. The presence of *S. typhi,* which is a major causative agent of typhoid fever[17], is of great concern and a threat to public health. Also, the contamination of salad vegetables with *Pseudomonas* spp. is a public health concern as it has been implicated in several human infections and spoilage of vegetables. The contamination with *Proteus vulgaris*, an opportunistic pathogen in humans and various animals implies the use of polluted water or cultivation on soil infested with such organisms[18]. The occurrence of *Bacillus subtilis* in vegetables in this study confirms its prevalence as one of the common bacteria that contaminate fresh vegetables[2,19]. Numerous fungi have also been indicted in the contamination of fresh fruits and vegetables. The presence of *Rhizopus stolonifer* and *Fusarium solani*, food-borne pathogens reported in this study is similar to the report of Akinyele *et al.*[16], who reported same fungi in fresh fruits and vegetables and their prevalent could be because of their adaptive nature, spore formers and are known as common environmental contaminants[19]. The presence of only *Ascaris lumbricoides* ova as the only geohelminths further explain its prevalence in Dutsin-Ma, which is similar to the findings of Auta *et al.*[20] and Nasiru *et al.*[21], who reported

Ascaris lumbricoides as the most common geohelminths that contaminate fruits and vegetables in Dutsin-Ma. The record of fruit and vegetable contamination by microorganisms of public health importance could be as a result of unhygienic practices in the cultivation, post-harvest handling, transportation and retailing practices at vendors' kiosks or stand.

CONCLUSION

This study reports that salad fruit and vegetables sold in Dutsin-Ma are contaminated with microorganisms of public health significance. *Staphylococcus aureus* was the bacteria with the highest occurrence, while *Rhizopus stolonifer* had the highest fungal occurrence. *Ascaris lumbricoides* were the only geohelminths recorded and only in lettuce. Lettuce was the most contaminated vegetable in this study. Adequate awareness of the public health implications that could result from the consumption of raw contaminated salad fruit and vegetables should be created. The promotion of hygiene among vendors handling these vegetables cannot be overemphasized. Regulatory agencies should ensure that hygiene standards are maintained by farmers and vendors in the cultivation, processing and distribution of salad fruits and vegetables.

SIGNIFICANCE STATEMENT

This study discovered and reported that salad fruit and vegetables sold in Dutsin-Ma were contaminated with pathogenic organisms of public health importance. Information provided here should guide vendors and consumers on handling salad fruits and vegetables. It also provides information to public health experts and organizations on possible means of infection and reinfection with these pathogens.

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