

RESEARCH NOTE

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Bacterial contamination of vegetables sold in Arba Minch Town, Southern Ethiopia

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Abstract

Objective: Unhygienically handled fruits and vegetables which are usually consumed in raw serve to transmit various infectious diseases. Bacteria are among the common vegetable contaminants. However, the species of contaminants and rate of contamination depends on various environmental and human factors. Hence, a cross-sectional study was conducted to assess the level of bacterial contamination and associated factors among vegetables marketed in Arba Minch town from January to March, 2018. A structured questionnaire was used to collect data regarding factors associated with bacterial contamination of vegetables. Selected vegetables were purchased and processed for examination of bacterial contamination by standard culture technique following standard protocols. All data were analyzed using SPSS version 20.0.

Results: A total of 347 vegetable samples were examined, of which 169 (48.7%) were positive for bacteria contamination. Cabbage (71.9%) was the most frequently contaminated vegetable. *E. coli* (31.4%) was the most frequent contaminant detected. Type of vegetables ($p = 0.000$) and market place ($p = 0.039$) show significant association with bacterial contamination. Bacterial contamination rate in the present study was significantly considerable. Therefore we recommend for the local health office to continuously monitor the contamination status of raw edible vegetables and take respective measures.

Keywords: Vegetables, Contamination, Bacteria

Introduction

Fruits and vegetables are highly beneficial components of balanced diet which play pivotal role for maintenance of health and prevention of disease [1]. They are rich in carbohydrate, vitamins, minerals and fiber contents [2–4]. As a result, the World Health Organization (WHO) recommended the intake of a minimum of 400 g of fruits and vegetables per day not only for the prevention of chronic diseases like heart disease, cancer, diabetes and obesity; but also for the prevention and alleviation of several micronutrient deficiencies [4, 5]. On the contrary, unhygienically prepared and consumed fruits and vegetables could bring potential risk of acquiring various infectious diseases. The risk of disease transmission is much

higher among fruits and vegetables consumed in raw and/or unwashed [2, 3, 6, 7].

Enteric bacterial infections are distributed virtually throughout the world and have been causing morbidity most commonly associated with infections in communities where poor environmental sanitation and personal hygiene are prevalent [8]. As much as 70% of diarrheal diseases in developing countries are believed to be of food borne origin [9, 10]. Vegetables can be contaminated with enteric bacteria of medical and public health importance during cultivation, harvest, transportation and further processing. As a result, they have been mentioned in many of previous food-borne outbreaks [11]. The poor personal and environmental hygiene and poor health system commonly observed in developing countries make the prevalence to be highest among the population in those countries [1, 5].

Enteric bacteria notably *Salmonella*, *Escherichia* and *shigella species* continue to be major global health problems and are the leading causes of morbidity and

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mortality in both developed and developing countries [9]. The increase in these food-borne infections may have resulted from increased consumption of contaminated fruits and vegetables [12].

Several factors may contribute to contamination of vegetables. Vegetables may get exposed to bacterial contaminants in the pre-harvest and post-harvest handling [13, 14]. Use of insufficiently treated wastewater for irrigation is an important risk factor for contamination of vegetables cultivated using irrigation in many of developing countries [13]. Contamination of soil with animal wastes and increased application of improperly composted manures to soil has also similar role [15]. Poor or inappropriate hygienic practice during production, transport, processing and preparation by handlers including consumers also contribute in vegetable contaminations [16].

If our target is to control infectious diseases, it is not enough to depend merely on chemotherapeutic intervention, but need the concerted effort to reduce and eliminate the potential sources of infection. To cope up with this effort, periodic detection of medically important infectious contaminants of vegetables and associated practices seek priority attention [17]. Therefore, the aim of the present study was to determine the level of bacterial contamination of selected vegetables and associated factors in Arba Minch Town, Ethiopia.

Main text

Methods and materials

Study design and area

A cross-sectional study was conducted from January to March 2018 in local markets of Arba Minch town, Southern Ethiopia. The town is located 454 kms away in the southern direction from Addis Ababa, capital city of Ethiopia. It is found at an altitude of 1200–1300 meters above sea level with an average annual temperature of 29.7 °C and rain fall of 900 mm [11]. The weather condition during our data collection period was hot and dry during January and February, and the minor rainy season starts in March. The climate of the town and surrounding rural area is conducive for cultivation of different fruits and vegetables. As a result, diverse types of fruits and vegetables are frequently utilized as sources of food in the town. Those fruits and vegetables are readily available in shops, super markets and open markets throughout the town and most types are eaten in raw.

Sample collection

A structured questionnaire administered to local vendors by face to face interview was used to collect data about factors associated with parasitic contamination of vegetables. About 200 gm of vegetables were purchased from four local markets (Sikela, shecha, Yet nebersh and

Konso sefer). Variable numbers of samples were collected from each vegetable type based on their abundance in markets. Accordingly, 100 *Lycopersicon esculentum* (tomato), 96 *Brassica oleracea* (cabbage), 66 *Capsicum annuum* (Green pepper), 62 *Daucus carota* (carrot) and 23 *Lactuca sativa* (lettuce) (totally 347) samples were collected. Samples were collected, put in sterile plastic bags, labeled, and transported through transporting cold box to the Medical Microbiology and Parasitology Teaching Laboratory of College of Medicine and Health Sciences for parasitological analysis. The Analysis was done within 24 h after collection.

Isolation of bacteria

Samples were transported in sterile packet and analyzed for isolation and identification of pathogenic bacteria following standard methods. About 25 gm of vegetable samples were rinsed thoroughly with sterile water and tenfold serial dilutions of each rinse water was made and 1 ml of 10^{-2} , 10^{-4} , 10^{-5} , 10^{-6} was pipetted into MacConkey, *Salmonella Shigella* agar, Manitol Salt agar and nutrient agar using the Pour Plate Technique. The plates were allowed to solidify, inverted and incubated at 37 °C for 24 h for colony formation. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Bacterial pathogens were isolated and identified on the basis of morphological, cultural and different biochemical tests following standard protocol as described elsewhere [18].

Statistical analysis

Both questionnaire and laboratory data were cleaned entered and analysed using SPSS version 20.0. The proportion of vegetables contaminated with different types of medically important bacteria were computed. Chi square test and logistic regression analysis were used to identify factors associated with bacteria contamination. Association between variables was considered statistically significant only if p-value ≤ 0.05 at 95% confidence level. The strength of association was measured through adjusted odds ratios.

Results

A total of 347 vegetable samples were collected from local markets and examined for bacterial contamination. Vegetables were collected from four markets namely Sikela (230, 66.3%), Shecha (57, 16.4%), Konso sefer (36, 10.4%) and Yetnebersh (24, 6.9%). Vegetables collected were Tomato (100, 28.8%), Cabbage (96, 27.7%), Green pepper (66, 19.0%), Carrot (62, 17.9%) and Lettuce (23, 6.6%). Results of this study showed that 169 (48.7%) vegetable samples were contaminated with bacteria. Cabbage was the most frequently contaminated vegetable (69, 71.9%)

followed by carrot (35, 56.5%). Four species of bacteria were detected *E. coli* being the commonest bacterial contaminant of vegetables (109, 31.4%) (Table 1).

The Chi square (X^2) test result show difference in bacterial contamination rate among different kinds of vegetables ($p=0.000$). Further binary logistic regression analysis showed that tomato was 2.244 (AOR: 2.244; 95% CI 1.112, 4.529) times more likely to be contaminated with bacteria as compared to green pepper. On the contrary cabbage was 73.5% less likely to be contaminated (AOR: 0.265; 95% CI 0.133, 0.529). Market place was also significantly associated with bacterial contamination ($p=0.039$). Vegetables from Sikela (AOR: 0.436; 95% CI 0.192, 0.991) and Shecha (AOR: 0.143; 95% CI

0.052, 0.394) were significantly protected from bacterial contamination compared to vegetable samples collected from Konso sefer (Tables 2 and 3).

Discussion

Bacterial contamination rate of vegetables in the present study was considerable. However, it is much lower than the same study result conducted in the same area before 9 years where all the vegetable samples examined were contaminated with *E. coli* [19]. Actually only tomato and lettuce were considered in the previous study and presence of bacteria other than *E. coli* were not considered. Data was collected in the dry season that the tendency of contamination due to flood was less in the present

Table 1 Frequency distribution of bacterial contamination among vegetables sold in local markets of Arba Minch Town from January to March 2018

| Vegetable type | Number examined | Number contaminated with each species of bacteria | | | | Total contaminated (%) |
|----------------|-----------------|---|------------------|----------------------|------------------------|------------------------|
| | | <i>E. coli</i> | <i>S. aureus</i> | <i>Shigella</i> spp. | <i>Salmonella</i> spp. | |
| Tomato | 100 | 20 | 4 | 0 | 9 | 29 (29.0) |
| Cabbage | 96 | 44 | 31 | 21 | 23 | 69 (71.9) |
| G. pepper* | 66 | 14 | 4 | 7 | 8 | 26 (39.4) |
| Carrot | 62 | 26 | 7 | 18 | 3 | 35 (56.5) |
| Lettuce | 23 | 5 | 0 | 3 | 3 | 10 (43.5) |
| Total (%) | 347 | 109 (31.4) | 46 (13.3) | 49 (14.1) | 46 (13.3) | 169 (48.7) |

Table 2 Chi square test of factors associated with bacterial contamination of vegetables sold in local markets of Arba Minch Town from January to March 2018

| Variables | Category | Number examined | Rate of parasite contaminated N (%) | X^2 | p-value |
|---------------------------|---------------------|-----------------|-------------------------------------|--------|---------|
| Vegetable type | Tomato | 100 | 29 (29.0) | 40.107 | 0.000 |
| | Lettuce | 23 | 10 (43.5) | | |
| | Cabbage | 96 | 69 (71.9) | | |
| | Carrot | 62 | 35 (56.5) | | |
| | Green pepper | 66 | 26 (39.4) | | |
| Washed before display | Yes | 49 | 27 (55.1) | 0.958 | 0.334 |
| | No | 298 | 142 (47.7) | | |
| Market | Sikela | 230 | 109 (47.4) | 8.346 | 0.039 |
| | Yetnebersh | 24 | 12 (50.0) | | |
| | Shecha | 57 | 36 (63.2) | | |
| | Konso sefer | 36 | 12 (33.3) | | |
| Means of display | On the floor | 338 | 166 (49.1) | 0.874 | 0.350 |
| | On table/shelf | 9 | 3 (33.3) | | |
| Handled by vendor who has | No formal edu | 36 | 15 (41.7) | 1.196 | 0.550 |
| | Primary school | 290 | 145 (50.0) | | |
| | Secondary/above | 21 | 9 (42.9) | | |
| Source of vegetables | Farmers | 260 | 120 (46.2) | 2.986 | 0.100 |
| | Large scale vendors | 87 | 49 (56.3) | | |

Table 3 Binary logistic regression of factors associated with bacterial contamination of vegetables sold in local markets of Arba Minch Town from January to March 2018

| Variables | Category | Number examined | Rate of bacteria contam N (%) | COR (95% CI) | p-value | AOR (95% CI) | p-value |
|---------------------------|---------------------|-----------------|-------------------------------|----------------------|---------|----------------------|---------|
| Vegetable type | Tomato | 100 | 29 (29.0) | 1.632 (0.845, 3.151) | 0.144 | 2.244 (1.112, 4.529) | 0.024 |
| | Lettuce | 23 | 10 (43.5) | 0.933 (0.361, 2.416) | 0.887 | 0.981 (0.369, 2.605) | 0.969 |
| | Cabbage | 96 | 69 (71.9) | 0.261 (0.134, 0.508) | 0.000 | 0.265 (0.133, 0.529) | 0.000 |
| | Carrot | 62 | 35 (56.5) | 0.514 (0.254, 1.042) | 0.065 | 0.542 (0.262, 1.121) | 0.098 |
| | Green pepper | 66 | 26 (39.4) | 1 | | | |
| Washed before display | Yes | 49 | 27 (55.1) | 1 | | | |
| | No | 298 | 142 (47.7) | 1.348 (0.735, 2.474) | 0.335 | | |
| Market | Sikela | 230 | 109 (47.4) | 0.555 (0.265, 1.163) | 0.119 | 0.436 (0.192, 0.991) | 0.047 |
| | Yetnebersh | 24 | 12 (50.0) | 0.500 (0.173, 1.441) | 0.199 | 0.574 (0.174, 1.898) | 0.363 |
| | Shecha | 57 | 36 (63.2) | 0.292 (0.121, 0.701) | 0.006 | 0.143 (0.052, 0.394) | 0.000 |
| | Konso sefer | 36 | 12 (33.3) | 1 | | | |
| Means of display | On the floor | 338 | 166 (49.1) | 0.518 (0.127, 2.106) | 0.358 | | |
| | On table/shelf | 9 | 3 (33.3) | 1 | | | |
| Handled by vendor who has | No formal edu | 36 | 15 (41.7) | 1.050 (0.353, 3.120) | 0.930 | | |
| | Primary school | 290 | 145 (50.0) | 0.750 (0.307, 1.834) | 0.528 | | |
| | Secondary/above | 21 | 9 (42.9) | 1 | | | |
| Source of vegetables | Farmers | 260 | 120 (46.2) | 1 | | | |
| | Large scale vendors | 87 | 49 (56.3) | 0.665 (0.408, 1.084) | 0.102 | 0.383 (0.196, 1.745) | 0.105 |

AOR, adjusted odd ratio; CI, confidence interval; COR, crude odd ratio

study. Cabbage was the most frequently contaminated vegetable in the present study (71.9%). This is in contrast to previous study in Arba Minch where tomato was predominantly contaminated. It is justifiable that cabbage has large surface area and course surface which enables to attach contaminants as compared to smooth surfaced vegetables with narrow exposed outer surface like tomato.

Among the four bacterial species identified, *E. coli* was the commonest contaminant (31.4%). This goes in line with findings from Istanbul (41.7%), Brazil (40–41.5%), Vietnam (100%) and Bangladesh (36%) [20–23]. *E. coli* is a fecal coliform bacterium usually excreted in stool that it is obvious to be abundant in the environment where open defecation is common (like Ethiopia). In addition, farmers usually use human excreta as a natural fertilizer; this accelerates rate of contamination of vegetables cultivated in such farms. However, the rate of *E. coli* contamination in this study is lower as compared to previous results from Istanbul, Brazil and Vietnam [20–22]. On the other hand, studies from Nigeria and Sudan indicated *Staphylococcus species* as the most frequent bacterial isolate [12, 24]. These differences might be due to variations in the time (season) of data collection and sensitivity of laboratory methods used.

The other three bacterial species detected have almost equal level of incidence; *S. aureus* (13.3%), *Shigella species* (14.1%), and *salmonella species* (13.3%).

Significantly higher contamination rate of *S. aureus* was observed in previous studies conducted in Bangladesh (24%), India (56–74%) and Nigeria (29.2%) [12, 23, 25]. *Salmonella species* were also more common contaminants than the present study result according to previous results from India (45–58%) [25]. None of the previous studies have reported *Shigella* separately. On the other hand a few studies have detected bacterial species like *bacillus*, *klebsiella*, *proteus* and *pseudomonas species* which we didn't report. All those variations might be due to differences in ecological abundance of the bacteria, culture techniques used and the time of data collection.

We have, probably for the first time, assessed factors contributing for or preventing from bacterial contamination of vegetables. Tomato was 2.244 times more likely to be contaminated with bacteria while cabbage was 73.5% less likely to be contaminated compared to green pepper. Based on the nature of the vegetables, leaves of cabbage seem to support many bacteria while the smooth surface of tomato less likely holds contaminant bacteria. We call up further study by recruiting equal number of each vegetable in order to drive definitive conclusion. The way vegetables are handled and displayed defers among markets so that the tendency to be contaminated also varies. Vegetables sold in Sikela and Shecha markets were 56.4% and 85.7% less likely to be contaminated by bacteria respectively compared to vegetables sold in Konso sefer.

Conclusion and recommendation

In conclusion bacterial contamination rate in the present study was significantly considerable. *E. coli* was the commonest bacterial contaminant. Cabbage was the most frequently contaminated vegetable. This alarms the public health sector to work on safe transportation, handling and utilization of contamination prone vegetables as well as continuous screening of on-market vegetables. We also recommend further study by increasing the sample size with equal number of samples from each item considering seasonal variations in order to exhaustively assess contributing factors.

Limitations

We didn't measure the intensity (colony count) of contamination due to lack on laboratory facility.

Authors' contributions

GA and MM conceived and designed the project, performed the experiment. GA, MM and MS analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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Acknowledgements

The authors thank Arba Minch University for giving ethical clearance. The authors' gratitude goes to Mr. Sefraye Batre for his kind participation during sample collection and laboratory analysis. The authors' regards also go to the vegetable vendors of Arba Minch town who participated in the study.

Competing interests

The authors declare that that have no competing interests.

Availability of data and materials

The original data for this study is available from the corresponding author.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical approval for the research was Granted by review board of Arba Minch University, College of Medicine and Health Science with a Project Code of Gov/AMU/TH.4.2./CMHS/MLS/04/09. Official permission letter was obtained from Arba Minch town administration office. Informed verbal consent was obtained from all participating vendors of vegetables.

Funding

Fund for data collection was obtained from Arba Minch University. No fund was obtained for designing of the study, result interpretation and manuscript writing.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 28 September 2018 Accepted: 26 October 2018

Published online: 30 October 2018

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