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Examination of Bacteria Contamination In Selected Fruits and Vegetables Sold in Uselu Market, Benin City, Edo State

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Abstract
Fruits and vegetables are essential for human health due to their nutritional qualities, but the consumption of raw foods leads to food safety problems as they are recognized as sources of transmission of infectious diseases. The aim of this work was to assess the bacteria contamination of some selected fruits and vegetables sold in Uselu market, Benin City, Edo State. A total of 175 fruits and vegetables were purchased from local vendors in Uselu market using different sterile polythene bags, properly labelled, and transported to microbiological laboratory. The samples were processed by culture methods and the isolates obtained were subjected to Gram staining and biochemical tests for identification. The sensitivity of the isolates was also tested using conventional antibiotics. A total of 141 (80.57%) were positive for bacterial contamination. Seven bacteria were isolated: *Staphylococcus aureus*, *Klebsiella spp*, *Streptococcus spp*, *Bacillus spp*, *Escherichia coli*, *Proteus miralbilis* and *Morganella spp*. The most prevalent bacterial isolate observed was *Staphylococcus aureus* (43.26%). Tomatoes had a higher frequency (14.9%) of bacterial contamination, with a significant difference (P = 0.0016). Single and mixed bacterial contamination have no significant association (P = 0.9806) with the infection. There was a statistically significant association between the risk factors and the infection (P<0.05). All the gram-positive bacteria were sensitive to tetracycline while majority of the gram-negative bacteria were sensitive to all the antibiotics used. The results further support public education on good personal hygiene and the dangers of consuming contaminated fruits and vegetables.

Keywords: Bacteria, Contamination, Fruit, Vegetables, Socio-economic factors

Introduction
Fruits and vegetables are highly beneficial components of balanced diet which play pivotal role for maintenance of health and prevention of disease (Okyay *et al.*, 2004). They are rich in carbohydrate, vitamins, minerals, and fiber contents (Gupta *et al.*, 2010; Nazemi *et al.*, 2012). 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 (Duedu *et al.,* 2014). 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 (WHO, 2009; Said, 2012; Ebrahimzedah *et al.*, 2013). Enteric bacterial infections are distributed virtually throughout the world and have been causing morbidity associated with infections in communities where poor environmental sanitation and personal hygiene are prevalent (Kusumaningrum *et al.*, 2003). 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 (Eni *et al.*, 2010). 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 (Wegayehu *et al*., 2013). Enteric bacteria notably Salmonella, Escherichia and Shigella species continue to be major global health problems and are the leading causes of morbidity and mortality in both developed and developing countries (Wegayehu *et al*., 2013). The increase in these food-borne infections may have resulted from increased consumption of contaminated fruits and vegetables (Mahvi and Kia, 2006). Several factors may contribute to contamination of vegetables. Vegetables may get exposed to bacterial contaminants in the pre-harvest and post-harvest handling (Erkan and Vural, 2008; Beuchat, 2018). Use of insufficiently treated wastewater for irrigation is an important risk factor for contamination of vegetables cultivated using irrigation in many of developing countries (Erkan *et al.*, 2008).

Contamination of soil with animal wastes and increased application of improperly composted manures to soil has also similar role (Mahvi and Kia, 2006). Poor or inappropriate hygienic practice during production, transport, processing and preparation by handlers including consumers also contribute in vegetable contaminations (Nazemi *et al.,* 2012). The spread of the bacteria is aided by bad hygiene, lack of good water, inadequate facilities and poor sanitation (Manjunath *et al.*, 2018). The use of sewage-contaminated water for irrigation, post-harvest handling, transportation and unsanitary practices in fruit and vegetable delivery all contribute to the contamination of vegetables (Jiang *et al*., 2017). The purpose of this study was to assess the level of bacterial contamination of some selected fruits and vegetables sold in Uselu market, Benin City, Edo State.

Materials and Methods

Study area

The Uselu Market in Benin City, Edo State served as the study site. Uselu Market is one of Benin City's most well-known markets. It is located in the Egor Local Government Area on Ugbowo Lagos Road. The Egor local government area, which spans 93 square kilometres, is situated in a tropical savannah. The region has two different seasons: the wet season and the dry season. The area's average temperature is 28 °C. Numerous towns and villages, including Okhoro, Uselu, Uwelu, Iguikpe, Ugbighoko, Iguediaye, Evbougide, and Oghedaivbiobaa, are part of the Egor local government region. According to the Ministry of Land and Survey (2008), 339,899 people live there. The market offers a wide range of products, including food items, apparel, textiles, meat, vegetables, plastics, kitchenware, cosmetics, drinks, and beverages.

Study design.

To ascertain the prevalence of bacterial contamination in fruits and vegetables available for sale at the Uselu market in Benin City, Edo State, a cross-sectional study was conducted between June and November of 2023.

Sample collection.

The following 10 fruit and vegetable kinds were purchased in the morning, from a few market vendors, between 8:00 and 9:00: Waterleaf (Talinum fruticosum), Oranges (Citrus sinensis), Watermelon (Citrullus lanatus), Carrots (Daucus carota), Green beans (Pisum sativum), Tomatoes (Solanum lycopersicum), Garden eggs (Solanum melongena), and Peppers (Capsicum annuum). Following proper labelling and collection in sterile polythene bags, the samples were sent to the University of Benin Microbiology laboratory for further processing. Once again, their identification was verified by a certified botanist from the Department of Plant Biology and Biotechnology at the University of Benin, Benin City, Edo State.

Sample processing.

Each of the ten selected fruit and vegetable samples, each weighing 200g, was cleaned individually in 200ml of ordinary saline solution before being inspected. The washing solution was let to settle for the whole night before any residual supernatant was disposed away. All specimens were aseptically injected into nutritious soup using discrete, aseptic swab sticks. It was then incubated for 24 hours at 28°C. The samples were then infected with selenite F broth and stored for 48 hours at 37°C. After a 24-hour interval, the inoculation nutrient broths were sub-cultured on MacConkey agar and the inoculated Selenite F broths on Deoxycholate agar (DCA). The plates were incubated at 37°C for twenty-four hours (Cheesebrough, 2009). Each isolate was identified using conventional biochemical and microbiological techniques in accordance with the recommendations given by (Barrow and Feltam, 2004; Garrity *et al.*, 2005; Cheesebrough, 2009).

Antibacterial susceptibility testing (AST)
Susceptibility testing was conducted using the Kirby-Bauer disk diffusion technique in compliance with the 2007 criteria set by the Clinical and Laboratory Standard Institute (CLSI). Test organism pieces were transferred to generate the inoculum using a sterile wire loop, and it was then suspended in sterile normal saline. The suspension density was adjusted by comparing it to a McFarland 0.5 Barium sulfate solution for standard opacity. A gradient of antibiotics that diffused into the Mueller-Hinton agar from impregnated paper disks was applied to the test organism, an oxoid, which was evenly distributed across the agar. At 37°C, the incubation period lasted 16–18 hours. The findings were categorized as sensitive or resistant using the CLSI, (2007) standardized table after measuring the diameter of the inhibition zones around the disks using a ruler to the nearest millimetre. The antibiotics used for Gram positive bacteria were Penicillin, Gentamycin, Chloramphenicol, Erythromycin, Ciprofloxacin, Tetracycline, and Ampicillin; for Gram negative bacteria, the antibiotics used were Gentamycin, Chloramphenicol, Tetracycline, Ciprofloxacin, Norfloxacin, Ceftriaxone, and Ampicillin.

Statistical analysis
Data obtained were analysed using Microsoft Excel for windows (2010 version) and SPSS version 17 as described by Ogbeibu (2005). Data was expressed in percentage when necessary and Chi-square test was carried out and differences were considered significant at values of $P < 0.05$.

Results
A total of 175 samples of fruits and vegetables were examined for bacterial contamination. Out of the 175 samples, 141(80.57%) were contaminated with bacteria (Fig 4.1). Seven bacteria of medical importance were identified: *Staphylococcus aureus* (43.26%), *Klebsiella spp* (26.95%), *Streptococcus spp* (4.96%), *Bacillus spp*. (7.80%), *Escherichia coli* (5.67%), *Proteus mirabilis* (9.22%) and *Morganella spp* (2.4%). *Staphylococcus aureus* was the predominant contaminated bacteria in the study with 61 (43.26%) (Table 1).

Table 2 shows the frequency of bacterial contamination among fruits and vegetables examined. Tomatoes had the higher prevalence 21(14.9%) of bacterial examined with a significant difference (P - 0.0016). The level of bacterial contamination of the fruits and vegetables showed that most of the samples were harbouring one or more bacteria isolates, with Tomato and Carrot having the highest 5(19.23%) mixed infection rate respectively. The rate of infection was not significantly associated (P=0.9806) (Table 3). There was statistically significant association between the risk factors and the infection ($P<0.05$) (Table 4). Gram positive bacteria (n=79) were tested against seven selected antibiotics. The results obtained showed that the organisms varied in their susceptibility to all the antimicrobials used. Chloramphenicol had the highest rate of resistance (30.4%), followed by Gentamycin (17.7%). All Gram positive bacterial tested were 100% susceptible to tetracycline (Table 5). Gram-negative bacteria (n = 62) were tested against selected 7 antimicrobial agents. Ampicillin had the highest rate of resistance (27.4%), followed by Gentamycin (12.9%). All Gram-negative bacteria tested were 100%susceptible to Norfloxacin (Table 6).

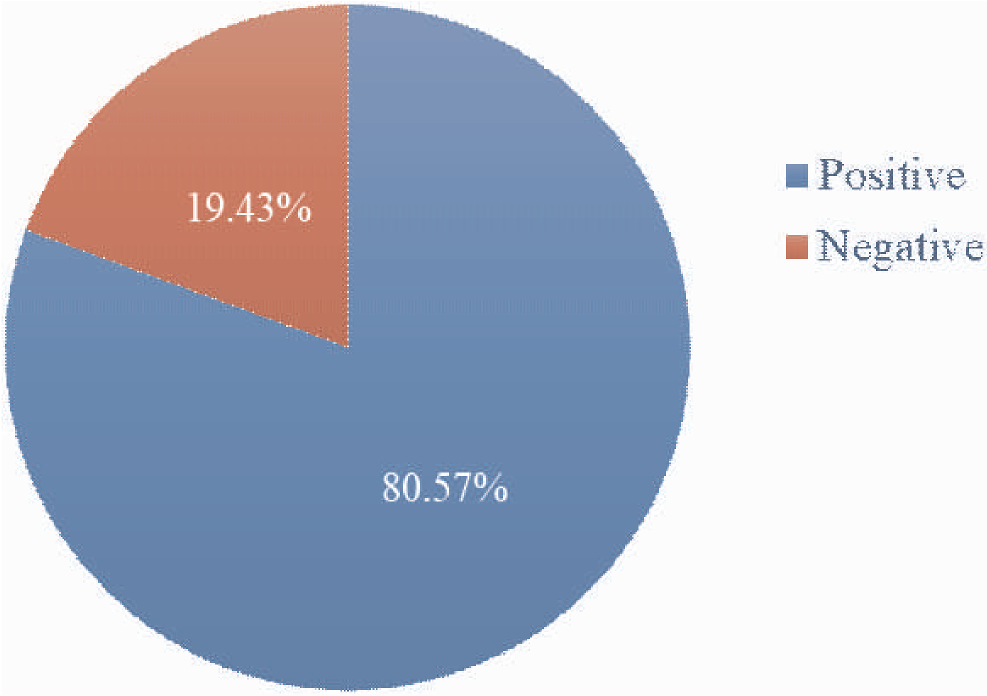

Figure 1. Prevalence of Bacteria Isolated from different varieties of fruits and Vegetables.

Table 1: Prevalence of Bacteria Isolated from selected Fruits and Vegetables

Bacteria	Number Isolated	Prevalence (%)
Staphylococcus aureus	61	43.26
Klebsiella spp	38	26.95
Streptococcus spp	7	4.96
Bacillus spp	11	7.80
Escherichia coli	8	5.67
Proteus mirabilis	13	9.22
Morganella spp	3	2.13
Total	141	80.57

Table 2: Frequency of bacteria contamination in the selected samples in the study area

Samples	Number Examined	Number Infected (%)	P - value
Pineapple	10	8 (5.7)	0.0016
Apple	10	6 (4.3)	
Orange	15	12 (8.5)	
Garden Egg	20	20 (14.2)	
Watermelon	10	3 (2.1)	
Tomato	25	21 (14.9)	
Pepper	25	20 (14.2)	
Carrot	20	18 (12.8)	
Pumpkin Leaves	20	15 (10.6)	
Green Beans	20	18 (12.8)	
Total	175	141 (80.57)	

Table 3: Prevalence of single and mixed Bacterial infections in the selected samples

Samples	Number Examined	Single Infection (%)	Mixed Infection (%)	
Pineapple	10	6 (5.22)	2 (7.69)	0.9806
Apple	10	5 (4.35)	1 (3.85)	
Orange	15	9 (7.83)	3 (11.54)	
Garden Egg	20	20 (17.39)	-	
Watermelon	10	3 (2.61)	-	
Tomato	25	17 (14.78)	4 (15.38)	
Pepper	25	15 (13.04)	5 (19.23)	
Carrot	20	13 (11.30)	5 (19.23)	
Pumpkin Leaves	20	12 (10.43)	3 (11.54)	
Green Beans	20	15 (13.04)	3 (11.54)	
Total	175	115 (65.71)	26 (14.85)	

Table 4: Prevalence of bacterial contamination among selected Fruits and Vegetables according to various risk factors

Variables	Number Examined	Number Infected (%)	OR	95% Cl	P-value
Educational level of vendors					0.0014
Primary	140	99			
Secondary	30	16			
Tertiary	5	0			
Sources of the produce					<0.0001
Farmers	62	15			
Middle men	90	80			
Private garden	23	2			
Market type					
Open market	165	115	48.030	2.759, 836.11	<0.0001
Grocery	10	0			
Means of display of produce					0.0002
On the floor	145	104			
On shelf	5	0			
On table	25	11			
Water source for washing					<0.0001
Tap	40	13			
Well	125	102			
Bore hole	10	0			
Wash before display					
Yes	98	47	0.122	0.055, 0.272	<0.0001
No	77	68			
Trimming of fingernails					
Yes	95	72	2.694	1.416, 5.125	0.0037
No	80	43			

Table 5. Antibiotic Susceptibility Pattern of Gram-Positive Bacteria Isolated from Fruits and Vegetables

	Rxn	Antimicrobial agents (%)						
		P	**CN**	**C**	**E**	**CIP**	**TE**	**AP**
Staphylococcus spp.	S	55 (90.2)	50 (82.0)	45 (73.8)	60 (98.4)	59 (96.7)	61 (100)	52 (90.2)
n=61	R	6 (9.8)	11 (18.0)	24 (26.2)	1 (1.6)	2 (3.3)	0 (0)	9 (9.8)
Streptococcus spp.	S	7 (100)	6 (85.7)	7 (100)	7 (100)	5 (71.4)	7 (100)	4 (57.1)
n=7	R	0 (0)	1 (14.3)	0 (0)	0 (0)	2 (28.6)	0 (0)	3 (42.9)
Bacillus spp.	S	9 (81.8)	9 (81.8)	11 (100)	11 (100)	9 (81.8)	11 (100)	7 (63.6)
n=11	R	2 (18.2)	2 (18.2)	0 (0)	0 (0)	2 (18.2)	0 (0)	4 (36.4)
Total	S	71 (89.9)	65 (82.3)	55 (69.6)	71 (89.9)	73 (92.4)	79 (100)	73 (92.4)
n=79	R	8 (10.1)	14 (17.7)	24 (30.4)	8 (10.1)	6 (7.6)	0 (0)	6 (7.6)

Key: S: Sensitive; R: Resistant; CN: Gentamicin; E: Erythromycin; C: Chloramphenicol; P: Penicillin; TE: Tetracycline; CIP: Ciprofloxacin AP: Ampicillin.

Table 6. Antibiotic Susceptibility Pattern of Gram-Negative Bacteria Isolated from Fruits and Vegetables

	Rxn	Antimicrobial agents (%)						
		CN	**C**	**NOR**	**CRO**	**TE**	**CIP**	**AP**
Klebsiella spp.	S	32 (84.2)	38 (100)	38 (100)	38 (100)	36 (94.7)	38 (100)	30 (78.9)
n=38	R	6 (15.8)	0 (0)	0 (0)	0 (0)	2 (5.3)	0 (0)	8 (21.1)
Escherichia coli	S	8 (100)	2 (25)	8 (100)	8 (100)	7 (87.5)	6 (75)	7 (87.5)
n=8	R	0 (0)	6 (75)	0 (0)	0 (0)	1 (12.5)	2 (25)	1 (12.5)
Proteus mirabilis	S	11 (84.6)	13 (100)	13 (100)	10 (76.9)	13 (100)	11 (84.6)	7 (53.8)
n=13	R	2 (15.4)	0 (0)	0 (0)	3 (23.1)	0 (0)	2 (15.4)	6 (46.2)
Morganella spp.	S	3 (100)	3 (100)	3 (100)	2 (66.7)	3 (100)	3 (100)	1 (33.3)
n=3	R	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	2 (66.7)
Total	S	54 (87.1)	56 (90.3)	62 (100)	58 (93.5)	59 (95.2)	58 (93.5)	45 (72.6)
n=62	R	8 (12.9)	6 (9.7)	0 (0)	4 (6.5)	3 (4.8)	4 (6.5)	17 (27.4)

KEY: S = Se nsitive R = Resistant; CN: Gentamicin; C: Chloramphenicol; NOR: Norfloxacin; CRO: ceftriaxone; TE: Tetracycline; CIP: Ciprofloxacin AP: Ampicillin

Discussion

Fruits and vegetables are the foundation of a nutrient-rich diet in fibre, vitamins, minerals, and other nutrients. Vegetables are made up of edible plant stems, leaves, and other plant parts, whereas fruits are the seed-bearing structure in flowering plants (Meeks, 2021).

This study has shown an overall prevalence of 80.57% of bacterial contamination in vegetable and fruit samples examined in this study. In contrast to this study, Erhirhie *et al*. (2020) reported a higher overall prevalence (62.5%) of bacterial contamination in Edo State, Nigeria. This disparity may be attributed to the cultivation in fields or orchards or during harvesting, post-harvest handling, refining and distribution, leading to the vegetables and fruits becoming infected with pathogenic microorganisms.

The prevalence of the isolates on ten (10) samples of vegetables and fruits decreased in the order of *Staphylococcus species* 43.26%, Klebsiella species 26.95%, *Proteus mirabilis* 9.22%, *Bacillus species* 7.80%, *Escherichia coli* 5.67%, *Streptococcus species* 4.96% and Morganella species 2.13%. Oluyege and Famurewa (2015) also reported the presence of *E. coli* (31.8%), *Klebsiella spp*. (19.4%), *Proteus spp*. (17.1%), Salmonella (14.0%), *Pseudomonas spp*. (12.4%), *Shigella spp*. (3.8%) and *Enterobacter spp*. (1.6%) from cooked foods sold in eateries in Ado-Ekiti, Nigeria. Similar to our study, Orunusi (2011) isolated similar organisms such as *S. aureus, E. coli, Enterobacter, Salmonella, Klebsiella, Pseudomonas aeruginosa, Proteus*, on street vended ready-to-eat fruits (pineapple and watermelon). The high bacterial contamination observed in the fruits and vegetables in this study could have been from the farmland, transportation from the farm to the markets or handling by the marketers. Also, it may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling (Abadias *et al*., 2008; Montville and Matthews, 2008).

Staphylococcus species (43.26%) was the most frequent bacteria isolated in this study. This corroborated the works of Ehimemen *et al*. (2019) who reported that *Staphylococcus aureus* was the most prevalent bacteria observed (80.0%) on fruits and vegetables sold in North - Western Nigeria. This is because *S. aureus* is a gram-positive bacterium frequently found on human nasal and skin microbiota, and can produce heat resistant enterotoxins, which are capable of damaging host cell membranes, causing foodborne diseases (Ho *et al.,* 2015). Also, the presence of *Staphylococcus species* in most fruits and vegetables is a reflection of poor sanitary condition adopted during cultivation, harvesting, transportation, storage and utilization or preparation process ranging from peeling, slicing, trimming, packaging, handling and marketing (Din*, et al*., 2011).

It was observed in this study that tomatoes was the most frequent contaminated with bacteria isolated (14.9%). This agrees to previous study in Arba Minch where tomato was predominantly contaminated (Wudneh, 2009). But in contrast to Alemu *et al*. (2018) that reported Cabbage was the most frequently contaminated vegetable (71.9%). 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 (Alemu *et al*., 2018). Watermelon had the least level of bacterial contamination because of its enclosed nature which makes it a bit difficult for bacteria to penetrate it. However, the contamination of the produce could have been from the vendor or the environment since the operating premises is usually kept unclean. The previous study of Akusu *et al*. (2016) on vegetable salads from street foods among different vendors in Port Harcourt metropolis in Nigeria agrees with the present study as high bacterial contamination was observed in some of the selected foods. Increase in the number of human infections and outbreaks is a resultant effect of the high rate in consumption of fruits and vegetables as most pathogenic or opportunistic bacteria inhabit them (Mashak *et al*., 2015).

Single infection was more prevalent (65.71%) compared to mixed infection (14.85%), Though this was not significantly different (p=0.9806). The possible explanation to the mixed infection might be the fact that in attempt to maximize profit, most venders combine contaminated fruits and vegetables with good ones during storage leading to the spread of bacteria from contaminated fruits to the good ones (Din*, et al***.,** 2011).

The significant association between the risk factors and the infection could be attributed to the fact that fruits and vegetables may be contaminated at any point in time. The sources of contamination can be grouped into two broader groups, namely, pre-harvest and post-harvest sources of contamination (Gil *et al*., 2015).

All the gram-positive bacteria were sensitive to tetracycline while majority were susceptible to the other antibiotics used. These findings are supported by the works of Michael *et al*. (2022) who reported that *S. aureus* was susceptible to Ciprofloxacin, ampicillin and cefuroxime. Srinu *et al*. (2012) also reported that *S. aureus* was sensitive to Ciprofloxacin. Among the gram-negative bacteria, majority were sensitive to all the antibiotics used. A significant number of *Klebsiella spp.* was resistant to gentamycin and ampicillin in this research. *E. coli* (75%) was resistant to Chloramphenicol while 25% was resistant to ciprofloxacin. This contrasts with the study of Aly *et al*. (2012) who reported that most *E. coli* isolates from food were sensitive to ciprofloxacin. Previous report by Srinu *et al*. (2012) examined *E. coli* was sensitive to ciprofloxacin and cotrimoxazole. Osterbald *et al*. (1999) reported 12% of *Escherichia coli* isolates from vegetable resistance to chloramphenicol. Disparities in antimicrobial susceptibility among bacteria can arise due to several factors. Inherent differences in bacterial species and strains play a crucial role in determining their susceptibility to antimicrobial agents. Bacteria vary in their genetic makeup, which can influence the presence or absence of specific antimicrobial resistance mechanisms, such as efflux pumps or enzymatic inactivation of antibiotics (Giedraitienė *et al*., 2011). Environmental factors, including exposure to antimicrobial agents in healthcare settings, agriculture, and the environment, can also drive the development and selection of resistant bacterial strains (Ahmad *et al*., 2021). It is important for vendors to maintain good hygiene practices to minimize the risk of contamination of fruits and vegetables sold on the streets with foodborne pathogens. Consumers are advised to thoroughly wash fruits with clean water before eating them. Both vendors and consumers should receive education about the consequences of foodborne pathogens in food to promote awareness and understanding.

Conclusion

This study suggests that microorganisms present in fruits and vegetables might cause food to rot and grow, rendering the fruits unsafe for human consumption. Tomatoes are one kind of produce that may accelerate the transfer of bacterial contaminations from the local supplier to the end user if they are handled and moved across marketplaces. Fruits and vegetables are often contaminated throughout the production, processing, storage, and selling processes. Therefore, farmers, consumers, and merchants would need to practice better personal cleanliness to reduce the occurrence of bacterial infection.

Recommendation

Environmental factors such as provision of adequate and safe water, introduction of basic sanitation practices for fruits and vegetables sellers in the market and immense governmental support must be involved if bacterial contamination must be tackled. To reduce the risk of illness, basic hygiene must be followed. It is crucial to keep in mind that when these fruits and vegetables were tested, they did not exhibit any indications of degradation. As such, it is not feasible to judge the quality of fruits and vegetables just based on their look; rather, fruits should always be cleaned and handled carefully before eating. It is strongly advised that irrigation, water quality, and the use of untreated night soil as fertilizer be regulated.

Conflict of interest

There is no conflict of interest.

Declaration

We attest that, neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere.

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