Coliform bacteria profile of the surface of raw salad vegetables sold in open markets in Owerrimetropolis,South Eastern Nigeria

ABSTRACT

The consumption of fresh vegetables has great health benefits. However, this has been linked to several food borne infections and disease outbreaks in the past. They are identified as agents in the transmission of human food pathogens. This study examined the coliform bacteria profile of raw salad vegetables sold in open markets in Owerri metropolis, South Eastern Nigeria.The samples of different raw salad vegetables (Cabbage, Cucumber, Carrot and Tomatoes) bought from different vendors in open markets were examined for the presence of coliform bacteria. Surface wash water of samples were used for the enumeration of Total and fecal coliforms. The results from the study showed that all the samples harboured coliform bacteria with the Cabbage recording the highest coliform count (CA_3= 2.8 x 10^7cfu/g on MacConkey agar) while the highest*E. coli* count occurred in Tomato (TO_3=2.7 x 10^6cfu/g on EMB agar);Carrot (CB_3= 2.6 x 10^4cfu/g on MacConkey agar)and Cucumber (CU_2= 3.9 x 10^2cfu/g on EMB agar) recorded the lowest coliform and *E. coli* counts respectively. The percentage prevalence of the isolates was*Enterobacter*species (83.3%), *Escherichia coli* (66.6%) and *Klebsiella* species (33.3%).The counts were obviously above the coliform acceptable limit (<100cfu/g) for salad vegetables. Positive detection of coliforms (especially, *E. coli*) is an effective confirmation of fecal contamination and highlights the importanceof observing good hygiene measures throughout the whole food processing chain. This could eventually help in reducing the microbial contents of the vegetables before consumption.

Keywords: serial dilution, coliform bacteria, inoculated, spread plate technique, prevalence, good hygiene

1. INTRODUCTION

"Vegetable usually refers to the fresh edible portion of certain herbaceous plants- roots, stems, leaves, flowers, fruits or seeds. They are either eaten fresh or prepared in a number of ways, usually as a savory, rather than sweet dish" [1]."Vegetables are rich in carbohydrates, antioxidants, minerals (especially, calcium and iron), vitamins (principally A and C) and fibres and often consumed

uncooked. Most fresh vegetables are low in calories and have water content in excess of 70%, with only about 3.5% protein and less than 1% fat" [2].Nutritionists emphasize the importance of raw vegetables in healthy diets, and researchers and governmental publicity campaigns around the world tend to recommend consumption of at least five servings of fruits and vegetables per day.

"Apart from the health benefits of consuming vegetables, the consumption of fresh vegetables has also been associated with risk for consumers"[3]."Vegetables, especially when consumed raw have been increasingly recognized as important vehicles for the transmission of human pathogens"[4]. [5] had also reported that "as fresh vegetables are eaten raw or slightly cooked to preserve the taste and their nutrient content, they serve as potential sources of various food borne infections and disease outbreaks.Consequently the increase in global consumption of fresh vegetablesis greatly threatened by an upsurge of microbial contamination"[6].

"Different agricultural practices can contaminate vegetables in different stages of production. Raw vegetables can become contaminated with coliforms some of which might be pathogenic while growing in fields, or during harvesting, post-harvesting, handling, processing and distribution"[7][8][9].
"Microorganisms that adhere to the surface of the vegetables are mainly coliforms that may survive even after washing and sanitizing steps due to the formation of biofilms on the surface of the vegetable or form protection by the cuticle of the vegetable"[10].

"The main issue associated with these products is the high microbiological risk associated with their consumption. Microbiological contamination is common and inevitable in vegetables growing in soil. Typical environmental microorganisms found in soil and irrigation water contaminate plants infiltrating through roots or exposed (wounded or cut) surfaces and get internalized by the plant's coating that creates a natural biofilm that protects them from surface treatments. The micro flora can be further modified by other microorganisms that come in contact with the product during each step of the production chain"[11][12].

The human infections associated with consumption of raw fruits and vegetables have increased during the past decade and as most of these produce are eatenwith minimal processing or without further processing, their microbial content may represent a risk factor for the consumer's health and therefore a food safety problem. Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with coliform bacteria contamination, particularly with members of the *Enterobacteriaceae*family.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh salad vegetables (cucumber, carrots, tomatoes and cabbage) were bought from five different markets located in Owerri, Imo State; namely- Ihiagwa, Naze, Irete, Obinze and ReliefMarkets. The vegetable samples wereput in separate sterile polythene zip lock bags and transported to the laboratory at temperature range 4–6 °C and analysed within 24hours of collection.

2.2 Preparation of Media

All media used were prepared according to manufacturer's specification. The media used includedNutrient agar, Eosin Methylene Blue Agar (EMBA) and MacConkeyagar(MA).

2.3 Isolation of Coliform Bacteria

Twenty-five grams of sample was immersed in 225 ml of sterile distilled water for 15 min, vigorously agitated and surface wash water used for analyses. Ten (10) folds serial dilution was made (for each sample) after vigorous shaking to suspend the sample.An aliquot portion (0.1 ml) of the 10^{-7} and 10^{-4} dilutions were spread plated ontoMacConkey Agar and EMBAgar respectively. The inoculums were evenly distributed with a sterile glass rod and incubated at 37^{0}C for 24 hours.Total colony forming units per gram was expressed as (cfu/g) after incubation[13].

2.4 Characterization and identification of coliform bacteria isolates

"The bacterial isolates were characterized using standard cultural (colonial), microscopic and biochemical methods. The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria" [14].

3. RESULTS

Table 1 shows the total heterotrophic bacterial counts from fresh vegetables on MacConkey agar plates and Eosin Methylene Blue Agar plates. From Table 1, it was observed that the highest Total Coliform Counts occurred in Cabbage (CB_3) and the highest *E. coli* Counts on Tomato (TO_3). The vegetable with the Lowest Total Coliform Counts was Carrot (CA_2) and cucumber had the lowest *E. coli* counts (CU_3).Table 2 represents the colonial, microscopic and biochemical characteristics of bacteria isolated from raw vegetables. The most probable microorganisms identified were *Klebsiella*sp, *Escherichia coli* and *Enterobacter*sp.Table 3 shows the distribution of bacterial isolates on the raw vegetable samples. From the table, it was observed that *Escherichia coli* and *Enterobacter*sp were present in all the four vegetable samples. Figure 1 represents the frequency of occurrence of the bacterial isolates. *Enterobacter*species have the highest occurrence among the bacterial isolates from the vegetable samples while *Klebsiella*species have the lowest occurrence.

Table 1 Total counts of bacteria isolated from raw vegetables

Sample codes	Location/Market	Total counts on MCA (cfu/g)	Total counts on EMBA (cfu/g)
Tomato (TO1)	Ihiagwa	4.0×10^6	2.9×10^3
Tomato (TO2)	Irete	5.7×10^6	1.7×10^3
Tomato (TO3)	Relief	6.9×10^6	1.7×10^3
Tomato(TO4)	Obinze	1.4×10^7	1.3×10^3
Tomato(TO5)	Naze	5.0×10^5	1.9×10^4
Carrot (CA1)	Ihiagwa	2.0×10^5	9.4×10^5
Carrot (CA2)	Irete	2.8×10^7	2.0×10^6
Carrot (CA3)	Relief	4.7×10^6	2.5×10^4
Carrot (CA4)	Obinze	1.9×10^6	2.5×10^5
Carrot (CA5)	Naze	2.8×10^6	2.1×10^5
Cucumber (CU1)	Ihiagwa	1.1×10^6	1.2×10^3
Cucumber (CU2)	Irete	1.6×10^7	3.9×10^2
Cucumber (CU3)	Relief	4.2×10^5	4.0×10^3
Cucumber (CU4)	Obinze	5.2×10^5	5.7×10^3
Cucumber (CU5)	Naze	4.5×10^6	6.9×10^4
Cabbage (CB1)	Ihiagwa	1.4×10^6	1.0×10^4
Cabbage (CB2)	Irete	5.3×10^5	5.0×10^3
Cabbage (CB3)	Relief	2.6×10^4	2.0×10^3
Cabbage (CB4)	Obinze	1.8×10^5	2.8×10^5
Cabbage (CB5)	Naze	1.4×10^6	1.1×10^3

Keys: MCA- MacConkey. EMBA- Eosin Methylene Blue Agar

Table 2 Colonial, Microscopic and Biochemical Characteristics of Bacteria Isolated from Raw Vegetables

Spo	Mot	Gram Rxn	Oxi	Cat	Coa	In	MR	VP	Suc	Lac	Glu	NO_3	Ure	Mal	Colonial characteristics	Identities of Organisms
-	-	-R	-	+	-	+	-	+	+	+	+	+	-	-	Moist and shiny purple metallic sheen colonies on EMBA and small smooth colonies on MCA	*Escherichia coli*
-	-	-R	-	+	-	-	+	-	-	+	+	+	+	-	Circular light pink colonies on EMBA	*Enterobacter*sp
-	+	-R	-	+	-	-	-	+	+	+	+	+	+	+	Small circular shiny and shiny slimy mucoid pink colonies on MCA	*Klebsiella*sp

Key:spo, spore stain;Mot, motility; Cat, catalase; Coa, coagulase; Oxi, oxidase; In, indole; MR, methyl red; VP, vogesproskaeur, Ure, urease production; $N0_3$, nitrate production; Glu, glucose; Suc, sucrose; Mal, maltose; Lac, lactose; -R, gram negative rod;

Table 3 Distribution of Bacterial Isolates on Raw Vegetable Samples

Samples	Bacterial isolates
TOMATOES	*Escherichia coli, Enterobacter*sp, *Klebsiella*sp
CUCUMBER	*Escherichia coli, Enterobacter*sp
CARROT	*Escherichia coli, Enterobacter*sp, *Klebsiella*sp
CABBAGE	*Escherichia coli, Enterobacter*sp

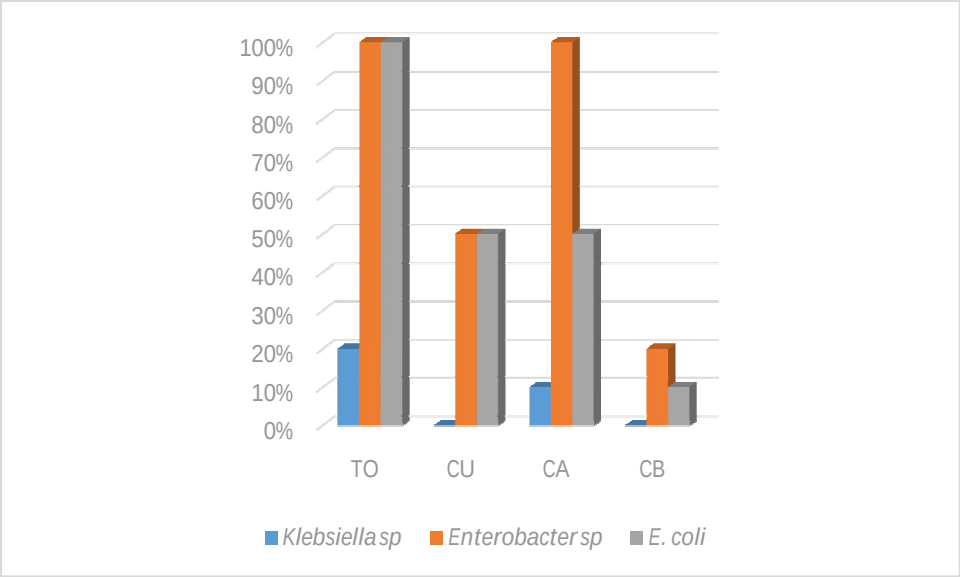

Figure 1 Frequency of Occurrence of Bacterial Isolates on Samples

4. DISCUSSION

"Salads are fresh vegetables which require minimal washing and processing and cut into desired shapes and sizes with knives or other shredding utensils and usually serve along with other foods including rice"[15]. "Worldwide, salad vegetables are considered a major source of nutrients for people and particularly as sources of cancer fighting agents for the skin"[16][17].

"The consumption of vegetables and vegetable products are vital for the total health of every individual, however, microbial contamination of these vegetables has become a serious challenge deserving of greater attention. Food safety problems continue to persist across the globe and remain a great challenge"[18].

The results from this study highlight the fact that the fresh vegetables sampled were all contaminated with coliforms and thus could possibly act as a transmission vehicle of many diseases. This conforms to the work of [19], who had also reported that coliforms were detected in all the raw salad vegetables sampled in their study. Vegetables such as salad vegetables, after cultivations from the farm pass through series of handling processes and preparations before they are taken to the marketor to consumers.This is a clear sign that coliform bacteria could be associated with raw vegetables sold in open markets and it should be of serious health concern [20]. This presence of coliform bacteria on these samples may also be attributed to the different unhygienic practices by food vendors such as poor handling practices during storage and at point of selling, as well as the sources of cultivation from which these vegetables were obtained. [21] had earlier reported that the raw materials for salad making often come into contact with soil and thus improper washing with water may result in high human health risk.

The organisms isolated from this study included *Klebsiella*species (33.3%), *Escherichia coli*(66.6%)and *Enterobacter*species (83.3%).This is in agreement with the work of [22], who reported 64% contamination of products of vegetable origin (pepper/ tomatoes sauce) with *E. coli* in Kumasi. In a related study,[23]reported the presence of *E. coli* in 66.6% of sampled ready- to-eat tomatoes sauce in the Tamale Metropolis. *E coli*load of ready-to-eat vegetables $>10^2$cfu/g is categorized as unwholesome for human consumption; [24] reported that'out of 30 salad vegetable *E. coli* were detected in 29 (96.7%) of them'. The presence of *E. coli*in food samples is an indication of faecal contamination and improper hygienic practices by food vendors [25]. Some strains of *E. coli* when present in food could cause gastroenteritis and diarrhoea in humans upon consumption. The presence of some opportunistic bacterial pathogens like*Klebsiella*and *Enterobacter*spp, in vegetables that are eaten raw either as epiphytic flora or as a result of contamination from soil, animal or human points out that fresh vegetables may represent a risk factor for infection in immune-compromised population, and therefore consumption of raw vegetables should be considered as a potential food safety concern in this population.

The highest fecal coliform counts were observed mostly in carrot samples similar to the report of [3]. Carrots being a root crop could have received contamination from the soil, irrigation water, animal wastes used as fertilizer, water used for washing and from handlers. Its pits and crevices retain dirt containing organisms which may not be easily removed by slight washing. Studies in Cameroon [26, 27, 3] reported that these vegetables are usually irrigated withwater polluted with faeces. The presence of all three types of bacteria in the vegetable samples is probably a reflection of the nature of the environmental conditions of the markets in this study [20]. During sample collection, it was clearly observed that the surroundings and places for vegetable salads display in the markets were not clean and tidy, and the handlers were not wearing hand gloves while the vegetables are being handled. Apart from these, the vegetable salads sold at markets sometimes have a long holding time, which could contribute to the accumulation of pathogenic bacteria [28]. This is, however, in variance with the work of [19], who recorded a low coliform

count in cabbage; hence the least contaminated.*Escherichia coli* and *Enterobacter*species were constantly isolated (most prevalent) in all the four vegetable samples.Populations of *E. coli* areoften measured for monitoring the sanitary condition of food and as an indicator of contamination of fecal origin [29].

Numerous disease outbreaks linked to contaminated fruit and vegetables have been summarized in recent reviews [30]. These outbreaks emphasize the effect that contaminated produce can have on human health. The risk of disease transmission is increased when fruit and vegetables are consumed raw.

During cultivation in fields or orchards or during harvesting, post-harvest handling, refining and distribution, vegetables become contaminated with pathogenic microorganisms [31]. At the market, microbial population levels can compromise the product quality by reducing its shelf-life, as well as pose a risk to consumer health [32].

Conclusion

The incidence of food borne illness is increasing each day in Nigeria.From the results obtained, all the salad vegetables were contaminated with coliform bacteria, which could be a threat to public health. However, the presence of pathogenic bacteria such as *E. coli* should not be underestimated, particularly when the vegetables are meant for raw consumption.In view of the above findings, more hygiene practices need to be adopted by vegetable vendors and consumers to minimize the risks of transmission of pathogens. The result can serve as an index for identifying microorganisms associated with particular vegetables. In addition, the government should have a better surveillance on the activities of vegetable vendors to minimize the risk of disease outbreak associated with consumption of contaminated salad vegetables.

REFERENCES

1.Britannica, T. Fresh Vegetables. Encyclopaedia Britannica. https://www. britannica.com/vegetable, 2021.

2. Santosa MI et al. Evaluation of minimally processed salads commercialized in Portugal. Food Control,2012; 23(1):275–281.

3. Weldezgina D. and Muleta D. Bacteriological contaminants of some fresh vegetables irrigated with Awetu River in Jimma Town, Southwestern Ethiopia. Adv Biol. 2016; 2016 Article ID 1526764, 11 pages

4. Ramesh, MN et al. Microwave blanching of vegetable. Journal of food science,2002;6**7**(1):390-398.
5. Yahia, E.M. Postharvest physiology and Biochemistry of fruits and vegetables. Woodhead Publishing. 2019: 1-17.
6. Septembre-Malaterre, A. et al. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: changes in bioactive compounds during lactic fermentation. Food ResearchInternational, 2018;104:86-99.

7. Sivapalasingam S, et al. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Protect, 2004;6**7**(10):2342–2353.

8.Abougrain AK, et al. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control,2010;**2**1(5):760–762.

9. Eraky MA. et al.Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. J Parasitol Res, 2014:1–7

10. Seo YH. et al. Microbial evaluation of minimally processed vegetables and sprouts produced in Seoul, Korea. Food SciBiotechnol,2010; 19(5):1283–1288.

11. Erickson, MC. Internalization of Fresh Produce by Foodborne Pathogens. Ann.Rev. Food Sci. Technol., 2012; 3: 283–310.

12. Meyer, KM. and Leveau, JHJ. Microbiology of the phyllosphere: A playground for testing ecological concepts.Oecologia,2012; 168: 621–629.

13. Sospedra I. etal.Survey of microbial quality of plant-based foods served in restaurants. Food Control, 2013;30(2):418–422.

14. Buchanan, R. E and Gibbon, N. E. *Bergeys Manual of Determinative Bacteriology*. Williams and Wilkens Company, Baltimore, USA.2000; 1246
15. Ababio FW, Lovatt P. A review on food safety and food hygiene studies in Ghana. 2014. Abankwa V, Grimard A, SomerKuria F (2009) United Nations Human Settlements Programme

16. Steinmetz KA and Potter JD. Vegetables, fruit, and cancer prevention: a review. JAm Diet Assoc.1996;96:1027–39

17. Coulibaly-KalpyJ,et al. Microbiological quality of raw vegetables and ready to eat products sold in Abidjan (Côte d'Ivoire) markets. Afr J Microbiol Res., 2017: 11(10): 204–210.
18. Ntuli V, et al. Microbiological quality of selected dried fruits and vegetables in Research,2017; 1–5.
19. Sujeet, K.M and Vipin, K. A study on prevalence of microbial contamination on the surface of raw salad vegetables. Biotech.,2017;**7**(13):1-9.
20. Eni, AO. et al.Microbial quality of fruits and vegetables sold inSango Ota, Nigeria.2012;4(5):291-296.

21.Akusu OM, et al. Microbiological quality of selected street vended foods in Port Harcourt metropolis, rivers state, Nigeria. Sky J Food Sci., 2016;**5**(2):008–11.

22. Boateng AE. Assessment of food hygiene practices by street food vendors and microbial quality of selected foods sold. A study at Dunkwa-On-Offin, Upper Denkyira East municipality of the Central Region. A thesis submitted to the department of community health, college of health sciences in partial fulfillment of the requirements for the degree of MSc. public health. KNUST. 2016.

23. Bonah E.Heavy metal health risk assessment and microbial quality of locally milled ready-to-eat tomato in tamale, Ghana. A thesis submitted to the institute of distance learning, Kwame Nkrumah University of science and technology in partial fulfillment of the requirements for the award of Master of Science degree (M.Sc. food quality management). KNUST. 2014.

24. Abakari, G., Jerry Cobbina, S and Yeleliere, E. Microbial quality of ready-to-eat vegetable salads vended in the central business district of Tamale, Ghana.International journal of food contamination.2018; **5**:3.

25. Bakobie N, et al. Microbial profile of common spices and spice blends used in tamale, Ghana. Int J Food Contam. 2017;4(10):1

26.MewouoYCM, et al. Microbiological quality of shallow irrigation water at Nkolbisson a sub-urban area of Yaounde (Cameroon): influence of some physicochemical properties. Int J Res Earth Environ Sci. 2014; 2(3):8–17.

27. Ntangmo TH, Temgoua E, Njine T. Physico-chemical and bacteriological quality of vegetable watering water in the Dschang Town, Cameroon. J Environ Protect. 2012;3:949–55.

28. Tunung, R. Prevalence and quantification of*Vibrio parahaemolyticus* in raw salad vegetables at retail level.2012; 20(2):391-396.

29. Erkmen O. Microbiology of Food. 3rd ed.: Efil Press, Ankara, 2013; 550 pp

30. Beuchat, LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect.2002;4:413–423.

31. Viswanathan, P. et al.Prevalence and growth of pathogens on salad vegetables, fruits and sprouts.2013;203(3):205-213

32.Beuchat, LR. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg lettuce and romaine lettuce using simulated commercial processing conditions.J. Food Protect.2004;67:1238-1242.