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Enteric Bacterial Contamination of Salad Vegetables Sold Within Anyigba, Kogi State, Nigeria

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ABSTRACT: This study evaluated the enteric bacterial contamination of cabbage, cucumber and carrots sold within Anyigba, Kogi State. These vegetables which are usually eaten raw in coleslaw and salads were analysed for the presence and level of enteric bacteria using standard microbiological procedures. Results revealed bacterial contamination of the vegetables with enteric bacteria from all the sales points. Mean bacterial counts recorded for theses vegetables from Good shepherd Hospital sale point, were 8.9×10^6, 1.03×10^7, and 4.85×10^6 cfu/g for cabbage, carrot and cucumber respectively. Mean bacterial counts recorded from University gate sale point were 1.08×10^6, 6.48×10^6, and 4.0×10^7 cfu/g and mean bacterial counts recorded from Garage market were 3.0×10^6, 1.21×10^7, and 1.25×10^6 cfu/g. There is no significant difference in the colony forming units of enteric bacteria from the different sale points ($P< 0.05$). Enteric bacteria isolated from the vegetables included *Escherichia coli*, *Enterobacter* spp, *Salmonella* spp, *Shigella* spp., *Proteus* spp, *Klebsiella* spp and *Serratia* spp. These results indicate that the vegetables are significantly contaminated with enteric bacteria and have poor microbiological quality that could potentially result in outbreak of foodborne illnesses. Contamination of these vegetables could be attributed to pre-harvest and post-harvest contamination sources.

Keywords: Enteric, Bacteria, Contamination, Foodborne, Illnesses.

Introduction

Salad is a term broadly applied to many food preparations that have a mixture of chopped or sliced ingredients which may be mainly vegetables (Harli and Franca 2003). Consumption of vegetable salad amongst the Nigerian populace have greatly increased in recent times. This can be attributed to the greater awareness of the nutritional health benefits of vegetables when included in diets. Vegetables have been cherished for their nutritional values. Apart from being rich in crude fibers, they contain water soluble vitamins including vitamin A and vitamin D. They also contain carbohydrates and minerals. Fresh produce, provide anti-oxidants and some other compounds that may lower the risk of cancer and other chronic diseases such as heart diseases, thus are important part of a healthy diet (Ofor *et al*., 2009).

Carrots, Cucumber and Cabbage are vegetables included in vegetable salads in addition to other vegetables or prepared as a combination of shredded raw cabbage and carrots dressed with cucumber as coleslaw. These vegetables get contaminated through pre-harvest and post-harvest means. Sources of pre-harvest contamination include irrigation water which may contain faecal matter, organic fertilizers used as manure and soil environment (Oluwaseun *et al*., 2018). Sources of post-harvest contamination include the equipment used in processing, the handlers, the processing environment, transport means, dust, rinse water (Sadiq *et al.,* 2015). A variety of bacteria are introduced to vegetables from these sources and some of them may be agents of foodborne illnesses when consumed along with the vegetables.

Gastroenteritis in several cases of humans have been attributed due to ingestion of contaminated vegetables in the last decade, with a rise in frequency (Kumar *et al*., 2021). Risk of contracting food-borne illness have mounted as consequence consumption of raw or improper cooked vegetables, and hence considered as well recognized medium for the spread of pathogenic enteric bacteria in human. Many epidemic incidences occur due to consumption of raw vegetables contaminated by pathogenic micro-organisms, such as *Salmonella* spp and *Escherichia coli* O157 (Manhique *et al*., 2020). Enteric bacteria particularly *E. coli* are commonly utilized as an indicator of hygiene, particularly in the case of faecal contamination. Their existence means that pathogens may be present as a result of human or animal faeces contamination. Others can be found in atmosphere (soil, nutrient-rich waters, rotting plant matter) and faeces as well, and some of them can also thrive in different water delivery systems. (Kumar *et al*., 2021)

The safety of ready to eat salads have been an issue of public health concern mainly because of the conditions under which they are grown, harvested, prepared and consumed. They can be contaminated with different types of foodborne pathogens from farm-to-fork (Garba et al., 2021). This becomes a serious concern as these vegetables are usually consumed raw or minimally heat treated to retain their nutrients. Vegetables have been associated with the outbreaks of food borne diseases in many countries due to exposure to pathogens (Liu, 2006).

Therefore, this study was aimed at enumerating enteric bacteria on carrots, cabbage and cucumber for surveillance and to create public awareness on the importance of proper washing and disinfection of salad vegetables before preparation and consumption.

Materials and methods

Study area: Anyigba is a town located in Dekina Local Government Area in Kogi State situated between latitude 7°15'N-7°29'N and longitude 7°11'E-7°32'E. With an average altitude of 385 metres above sea level and total land mass of 420 sq.km² and has an estimated population of 189,976 persons (Ifatimehin *et al*., 2014)

The land use of Anyigba is predominantly agrarian in the first instance but is fast changing because of the transformation initiated in the economic landscape by the presence of Kogi State University which has become a rallying point both for education and commerce.

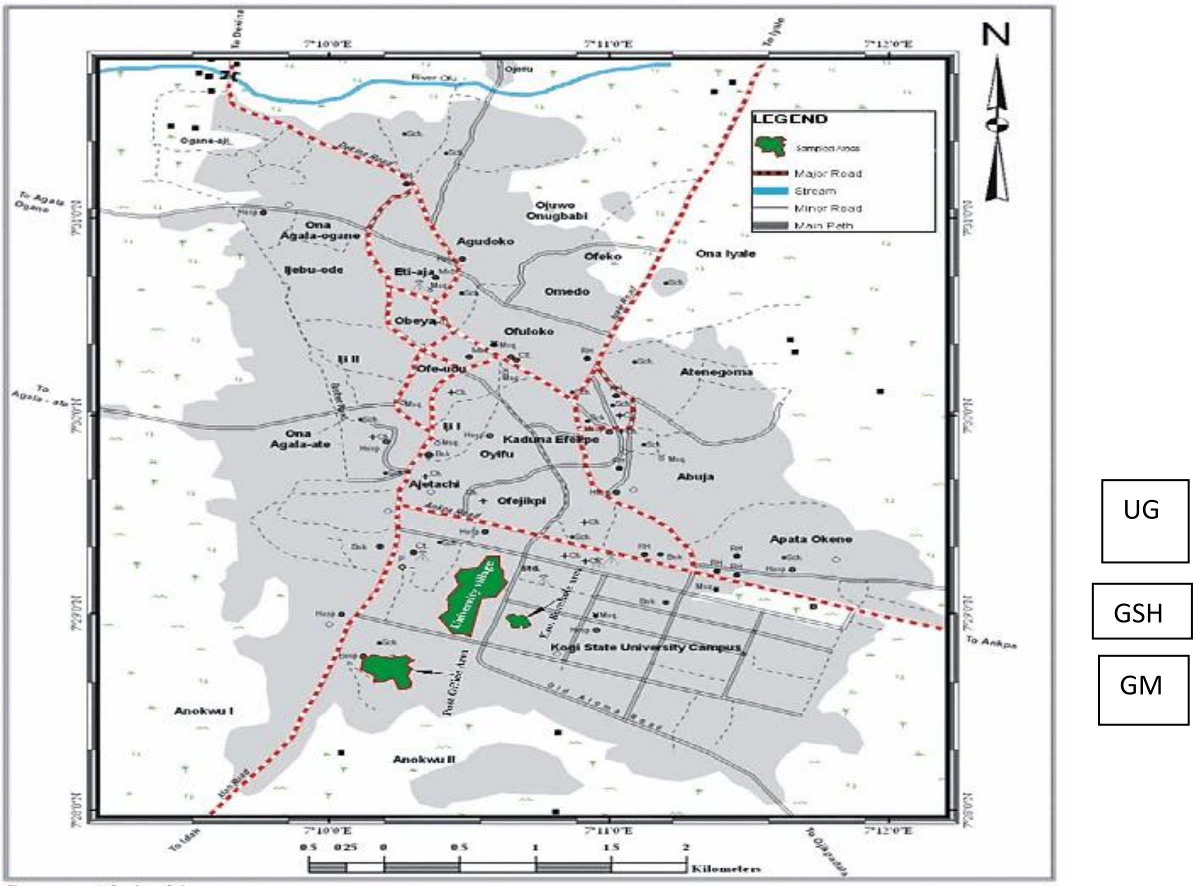

Fig. 1: Map of Anyigba and location of sales point of vegetables. (Ifatimehin *et al* 2014).

Sample collection: Fresh samples of cabbage, carrots and cucumber were collected from three different sales point within Anyigba for microbiological analysis. Samples were collected on a weekly basis in the months of August and September. Samples were transported immediately after purchase in well-labelled, sterile plastic containers with cover to the Microbiology laboratory of the Prince Abubakar Audu University Anyigba.

Microbiological analysis

Preparation of sample: For carrots and cucumber, ten grams of each sample were aseptically peeled with clean sterile scalpels and transferred into sterile 250ml beaker containing 90mls phosphate buffered saline (PBS). Beakers containing the samples were thoroughly shaken to dislodge bacteria into the PBS. Five outer leaves of the cabbage samples were aseptically shredded into 90mls of PBS in sterile beakers and also shaken as the beakers containing the carrot and cucumber samples. Serial dilutions of the resulting liquid from the washed vegetables (Kumar *et al*., 2021) were made until dilution 10^6 was obtained. One ml of the respective dilution was subsequently used as inoculum.

Isolation of enteric bacteria: Eosin methylene blue (EMB) agar was used for the primary isolation of enteric bacteria from the vegetables. Other media used in the course of analysis were Salmonella- Shigella agar, Triple Sugar Iron (TSI) agar, Nutrient agar (NA), Urease Agar Base, Simon's citrate Agar. All these media were prepared according to manufacturer's instruction for the purpose of isolating the enteric bacteria in pure cultures and also for some biochemical tests.

Inoculated Petri dishes were incubated at 37 °C for 24 h. The number of colonies were counted and recorded. Colony forming unit per gram of vegetable was calculated with this formula:

CFU/g = no. of colonies × reciprocal of dilution factor.

Cultural characteristics of the colonies arising from the incubation were described according to the criteria described by Cheesebrough (2000). Microscopic observation of Gram- stained slides prepared from typical colonies was done for the cell shapes, sizes and arrangement of the bacterial cells. Typical colonies were obtained as pure cultures and used for standard biochemical tests. Tests conducted for the presumptive identification of enteric bacteria were catalase, indole, TSI agar (for sucrose, lactose and glucose utilization, acid and gas formation and hydrogen sulphide production), citrate and urease (Pesewu *et al*., 2014).

Chi square was used to analyse significant difference in the colony forming units of the bacteria on the salad vegetables and also the frequency of isolation of the bacteria.

Results

Table 1 shows the enteric bacteria load of cabbage, carrots and cucumber sampled from three (3) strategic locations in Anyigba. Cucumber obtained from the Good Shepherd Hospital sales point had the highest bacterial count of 4.0x 10^7 cfu/g. While the lowest was recorded in cabbage sampled from University gate sales point with 1.08 x 10^6 cfu/g.

The cultural and biochemical characteristics employed in the presumptive identification of the bacteria isolated from the vegetables are presented in Table2. The identified isolates are *E. coli*, *Enterobacter* spp, *Shigella* spp, *Proteus* spp, *Salmonella* spp, *Klebsiella* spp and *Serratia* spp. The frequency of isolation of enteric bacteria is illustrated in Fig. 2. Seven genera of enteric bacteria were isolated. Most commonly occurring amongst them is *Enterobacter* species (100%). This genus of bacteria was isolated from all the vegetables at every time of sampling. This was followed by *E. coli* which occurred 100% in cabbage and carrots and 83.33% in cucumber. The least isolated was *Shigella* species. It had 16.67% and 8.33% in cabbage and carrots respectively. While it was not isolated (0.0%) in Cucumber.

Table 1: Mean Colony forming units (CFU/g) of enteric bacterial isolates from salad vegetables sold in Anyigba. (N=36)

Location/sales point	**Cabbage (n = 12)**	**Carrots (n = 12)**	**Cucumber (n = 12)**
GSH	8.90×10^6	1.03×10^7	4.85×10^6
UG	1.08×10^6	6.48×10^6	4.0×10^7
GM	3.0×10^6	1.21×10^7	1.25×10^6

Key: GSH = Good shepherd Hospital sale point, UG = University gate sale point, GM = Garage market sale point

Table 2: Cultural and biochemical characteristics of enteric bacterial isolates from salad vegetables (EMB agar)

Isolate no.	Cultural characteristics	Gram reaction	Cell shape	cat	cit	urease	indole	triple agar test slope/butt H_2S	sugar	iron gas	Presumptive identification
1	Spindle, Punctiform, purple, metallic sheen	-ve	Short rods	+	_	-	+	Y/Y	+	-	*E. coli*
2	pink, moist, raised, smooth, 6mm	-ve	Short rods	+	+	-	-	Y/Y	+	-	*Enterobacter spp*
3	pale, translucent, smooth, 2mm	-ve	Short rods	+	+	-	-	R/Y	-	-	*Shigella spp*
4	pale, translucent, flat, large	-ve	Short rods	+	+	-	-	R/Y ++		+	*Salmonella spp*
5	Pale pink, large, spreading irregular	-ve	rods	+	+	+	-	R/Y +		+	*Proteus mirabilis*
6	Pink, dark centered, mucoid	-ve	rod	+	+	+	+	Y/Y	+	-	*Klebsiella spp*
7	Pink, raised, moist	-ve	rod	+	+	-	-	R/Y	+	-	*Serratia spp*

Key: cat (catalase test), cit (citrate test), -ve (Gram negative), +ve (Gram positive), +(positive), - (negative), H_2S (hydrogen sulphide), Y (yellow), R (red), EMB (Eosin Methylene Blue)

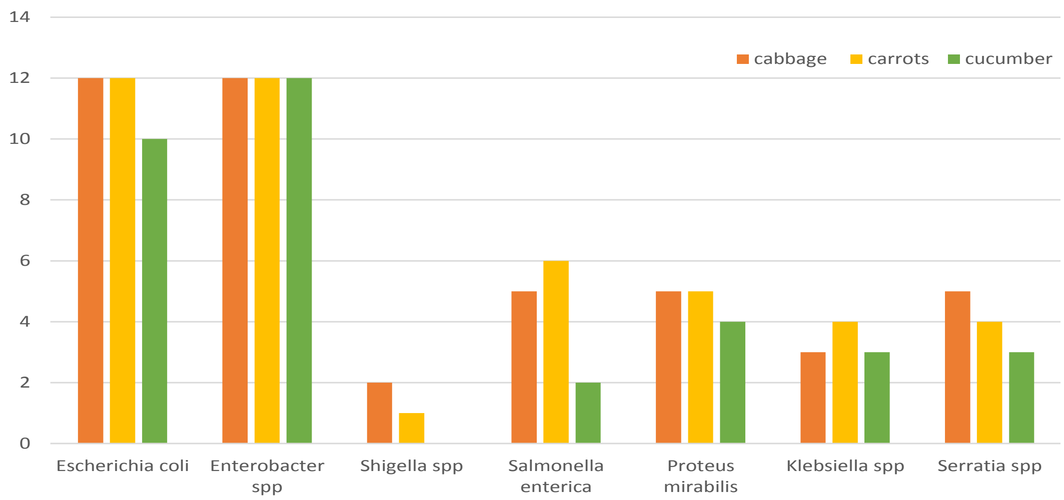

Figure 2: Frequency of isolation of enteric bacteria from the three salad vegetables

Discussion

The results obtained in this study shows that cabbage, carrots and cucumber are contaminated with enteric bacteria belonging to different genera. This is consistent with the results of Itohan *et al*., (2014) who isolated *E. coli*, *Enterobacter spp*, *Salmonella spp and Shigella spp* from salad vegetables in Abuja, Nigeria. The colony forming units of bacteria obtained from their study appears to be higher than the ones obtained in this study. This can be attributed to the medium used for the primary isolation of bacteria from the vegetable samples. This study employed the use of MacConkey agar which is inhibitory to most Gram-positive bacteria. The frequency of occurrence of *E. coli* is in agreement with Abakari *et al*., (2018) who reported the occurrence of 96.7% *E. coli* from 30 salad samples vended in central business district of Tamale Ghana. *E. coli* and *Salmonella* are among the most important pathogens of concern to produce safety (Garba *et al*., 2021). In a similar study also in Ghana, coliforms were isolated in 100% out of 100 samples analysed. The identified species included *Klebsiella* spp., *E. coli*, *Citrobacter* spp. and *Enterobacter cloacae* (Ghimire *et al*., 2020)

The high percentage of incidence of *Enterobacter* spp. and *E. coli* in raw vegetable samples may be due to one or all of these factors; faecal contamination from animal manures, irrigation water, cross contamination by food handlers through poor hand washing, or contamination of utensils. Pesewu *et al*., 2014 also reported that the presence of *E. coli* on the salads samples investigated may be as a result of faecal contamination because the bacterium is present in sewage, faeces, soil, water, and commonly come in contact with vegetables as result of the water used during the growing processes of the vegetables. Soil and irrigation water are known to be primary sources of hazardous microbiological contaminants in the production environment. Treatment of soil amendments such as animal fertilizers by physical or biological means is known to inactivate a wide range of potential foodborne pathogens that could be transferred to growing crops (FAO,2023). Composting, a widely practiced treatment for animal fertilizers, has been shown to reduce the transfer of E. coli from amended soil to growing lettuce plants (Chukwu *et al*., 2022). Treatment of soil amendments is mandated or recommended for leafy vegetable production in many jurisdictions. Additional control may be achieved by the use of physical barriers to prevent contact between soil and edible portions of a growing plant. For example, plastic mulch was shown to reduce the transfer of Salmonella from contaminated soil to growing lettuce in laboratory scale experiments (Honjoh *et al*., 2014). Cultivation of leafy vegetables in plastic mulch beds, a common agronomic strategy in some production systems, may contribute to risk reduction.

The isolation of enteric bacteria from salad vegetables and the numbers recorded are a warning signal to the fact that improperly washed and processed salad vegetables can serve as route of transmission of gastrointestinal diseases which have the potential of transforming to issues of public health concerns like epidemics if not properly controlled.

Faecal contamination from animal manures, irrigation water, cross contamination by food handlers through poor hand washing, or contamination of utensils are the most likely sources of these enteric bacteria on salad vegetables. It is therefore advisable to properly disinfect these vegetables by thoroughly washing them with clean portable water before preparation. Addition of a small quantity of cooking salt to the water can also drastically reduce the microbial load of these vegetables Preventives measures to reduce bacterial contamination of vegetables from farm to fork should be continuously made public to decrease incidences of foodborne illnesses arising from contamination of this group of foods which are either not heat treated or minimally heated to ensure their nutrients are not lost.

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