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SHORT COMMUNICATION

Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria

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ABSTRACT

Salad vegetables are essential part of people's diet all around the world. They are usually consumed raw and often without heat treatment or thorough washing; hence have been known to serve as vehicles for the transmission of pathogenic microorganism associated with human diseases. Fresh samples of lettuce, carrot and cucumber collected from different markets and vendors in Abuja Municipal Area Council, Federal Capital Territory, Nigeria were evaluated for bacterial loads using spread plate agar dilution method. Bacterial loads ranged from 1.6 x 10^6 to 2.9 x 10^8 cfu/g. *Escherichia coli*, *Klebsiella* and *Enterobacter* were amongst the coliforms (lactose fermenters), while *Proteus*, *Pseudomonas aeruginosa*, *Salmonella* and *Shigella* were non-lactose fermenters associated with the samples. *Staphylococcus aureus* was isolated from majority of the samples.

Keywords: salad vegetables, heat treatment, coliforms, pathogenic, bacterial load, agar dilution

INTRODUCTION

Vegetable salad is a very common food accompaniment in Nigeria. The vegetables that usually make up this recipe include tomatoes, cucumber, carrots, green chili, cabbage and lettuce. They are sold in almost every market, and can be seen hawked around by traders. Fruits and vegetables have been identified as significant sources of pathogens and chemical contaminants (Uzeh *et al*., 2009). As a result, environmental and food microbiologists have continued to identify and suggest control measures for hazards at all stages in the supply chain (Johngen, 2005).

Khan *et al*. (1992) reported that bacterial contamination results from various unsanitary cultivation and marketing practices. In another study, Tambekar *et al*. (2006) reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any heat treatment. These vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling, harvesting equipments, transport containers, wild and domestic animals. Pathogens from the human and animal reservoir as well as other environmental pathogens can be found at the time of consumption. Although spoilage bacteria, yeasts and mould dominate the micro flora on raw fruits and vegetable, the occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has also been documented (Hassan *et al*., 2006).

Coliforms are facultative anaerobic Gram negative rods belonging to the family *Enterobacteriacaea*. They are known contaminants of food and water, causing various intestinal and extra-intestinal infections such as urinary, central nervous system and respiratory tract infections (John, 2007). The presence of *E. coli*, *Enterobacter* sp., *Salmonella* sp., *Shigella* sp and *Pseudomonas aeruginosa*, has been reported in salad vegetables (Khan *et al*., 1992; Tambekar, 2006). Mehmet and Aydin (2008), also reported the presence of *E. coli* in some green leafy vegetables.

Due to the favourable climatic condition for cultivation of salad vegetables, as well as the cultural practice of dwellers in Northern Nigeria, the consumption rate of these vegetables is higher than in other regions in Nigeria. This study was therefore designed to assess the bacteriological profile of some salad vegetables sold in Abuja Municipal Area Council (AMAC), Federal Capital Territory (FCT), Nigeria.

MATERIALS AND METHODS

Collection of samples

A total of 15 samples of carrots (5), cucumber (5) and lettuce (5) were collected in sterile polythene bags from different markets and vendors in AMAC, Federal Capital Territory, Nigeria as shown in Table 1.

Isolation and identification of bacteria by spread plate method

A 25 g of vegetable samples were weighed, rinsed in a 250 mL beaker containing 100 mL of sterile distilled water

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and rinsed water samples were diluted $1:10^{-2}$ and $1:10^{-4}$. 0.1 mL of each dilution was spread on MacConkey agar (Oxoid, England) and the plates were incubated at 37 °C for 24 h for isolation of bacteria (Khan *et al*., 1992). Total viable counts were determined by counting both red and non-red colonies growing on the plates. Based on their morphological characteristics, red and non-red colonies were selected from each MacConkey agar plate for Gram staining, biochemical tests namely: IMViC (Indole, Methyl Red, Voges-Proskauer, citrate), urease, oxidase, catalase, triple sugar iron; and sub-culture on differential media (eosin methylene blue agar at 37 °C and 44 °C for 24–48 h; and mannitol salt agar, and cetrimide agar at 37 °C), nutrient agar (Holt *et al*., 1994).

RESULTS

Total viable counts

The salad vegetables showed a wide variation in total viable count ranging from 1.6 x 106 to 2.9 x 108 cfu/g at 37 °C (Table1).

Cultural characteristics and biochemical Identification of isolated strains

A total of 22 bacterial types were isolated. Non-red colonies from MacConkey plates that grew with golden yellow on mannitol salt agar and were Gram positive, coagulase-positive and catalase-positive were taken as *Staphylococcus aureus*; non-red colonies from MacConkey plates that grew with greenish pigment on nutrient and on cetrimide agar, and were Gram negative, indole-negative, oxidase positive and coagulase-negative were taken as *P. aeruginosa*; non-red colonies from MacConkey plates that were Gram-negative, indole-negative, methyl red–positive, Voges-Proskauer-negative, citrate-negative, acidic butt, alkaline slant with no blackening on TSI slant were taken as *Shigella* spp., non-red colonies from MacConkey plates that were Gram negative, indole-negative, methyl red–positive, Voges-Proskauer-negative, citrate-positive, acidic butt and alkaline slant with blackening on TSI slant, urease-negative and colourless colonies with black center on SS agar were taken as *Salmonella* spp., non-red colonies from MacConkey plates that were Gram negative, indole-negative, methyl red–positive, Voges-Proskauer-negative, citrate-positive, acidic butt and alkaline slant with blackening on TSI slant and urease-positive were taken as *Proteus* spp. Red colonies from MacConkey plates that grew with greenish metallic sheen on EMB agar and were Gram negative, indole-positive, methyl red-positive, Voges-Proskauer-negative and citrate-negative were taken as *E. coli*; red mucoid colonies from MacConkey plates that were Gram negative, indole-negative, methyl red-negative, Voges-Proskauer-positive and citrate-positive were taken as *Klebsiella* spp.; red colonies from MacConkey plates that were Gram negative, indole-negative, methyl red-negative, Voges-Proskauer-positive, citrate-positive and urease-positive were taken as *Enterobacter* spp. The genera of the bacteria isolated and the percentage occurrences are *S. aureus* (46.7%), *Klebsiella* spp. (26.7%), *Enterobacter* spp. (20.0%), *Proteus* spp. (13.3%), *and P. aeruginosa* (13.3%), *E. coli* (6.7%), *Shigella* spp. (6.7%) and *Salmonella* spp. (6.7%).

DISCUSSION

Freshly consumed vegetables especially those used in salad mixtures, have been implicated in food poisoning and thus hazardous to the health of the consumers. This could be linked to the fact that most of these vegetables are consumed without being subjected to thermal process or even thorough washing (Lund, 1992).

Table 1: Total viable count of salad vegetables.

Sampling No	Date of Sampling	Site of Sampling	Type of Sampling	Total Viable Count (cfu/g)
1.	27/04/09	Lugbe	Lettuce	8.6×10^7SD 8.49×10^6
2.	27/04/09	Lugbe	Carrots	4.4×10^7 SD 5.66×10^6
3.	27/04/09	Lugbe	Cucumber	2.4×10^7 SD 2.83×10^6
4.	04/05/09	Wuse	Lettuce	2.9×10^8 SD 1.70×10^7
5.	04/05/09	Wuse	Carrots	4.6×10^7 SD 2.83×10^6
6.	04/05/09	Wuse	Cucumber	7.6×10^6 SD 5.65×10^5
7.	10/05/09	Garki	Lettuce	6.4×10^7 SD 1.13×10^5
8.	10/05/09	Garki	Carrots	2.4×10^7 SD 0
9.	10/05/09	Garki	Cucumber	1.8×10^7 SD 2.83×10^6
10.	18/05/09	Karimo	Lettuce	8.4×10^7 SD 5.66×10^6
11.	19/05/09	Karimo	Carrots	1.0×10^8 SD 0
12.	18/05/09	Karimo	Cucumber	1.5×10^7 SD 1.13×10^6
13.	24/05/09	Kuchingoro	Lettuce	1.2×10^8 SD 5.66×10^6
14.	24/05/09	Kuchingoro	Carrots	1.6×10^8 SD 0
15.	24/05/09	Kuchingoro	Cucumber	1.6×10^6 SD 5.66×10^5

Key: SD = Standard deviation

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Table 2: Presence of pathogenic bacterial flora on vegetables.

Vegetables	No. of samples	*Proteus* spp.	*E. coli*	*Enterobacter* spp.	*P. aeruginosa*	*S. aureus*	*Salmonella* spp.	*Shigella* spp.	*Klebsiella* spp.
Lettuce	5	1	1	1	-	3	1	-	2
Carrots	5	1	-	1	1	2	-	-	1
Cucumber	5	-	-	1	1	2	-	1	1
Total	15	2	1	3	2	7	1	1	4
	%	13.3	6.7	20	13.3	46.7	6.7	6.7	26.7

In this study, the total viable count was done on MacConkey agar (Oxoid, England) by spread plate count method (Khan *et al*., 1992). Among the carrots analyzed, samples sourced from Kuchingoro had the highest total viable count of 1.6 x 10^8 cfu/g. This was higher than the total viable count of 5.7 x 10^6 cfu/g for carrots samples reported by Uzeh *et al*. (2009). Hall *et al*. (1967) suggested that a limit of 10 cfu/g should be standard with market raw food. Carrots are usually harvested from the soil hence can become contaminated by pathogenic organisms in soil.

Among the three different salad vegetables analyzed, cucumber samples had the lowest bacterial load. The highest total viable count for cucumber samples was gotten from samples sourced from Lugbe with a load of 2.4 x 10^7 cfu/g while those sourced from Kuchingoro had the lowest load of 1.6 x 10^6 cfu/g. This result was comparable to the bacterial load of 1.9 x 10^6 cfu/g reported by Abdullahi *et al*. (2010). Unlike carrots, cucumber fruits are rarely contaminated by soil pathogens as they do not come in contact with soil. Contamination with these pathogens could be due to poor hygiene practices by handlers.

Lettuce samples sourced from Kuchingoro had the highest coliform load of 2.9 x 10^8 cfu/g. This was higher than a load of 6.9 x 10^6 cfu/g reported for lettuce samples by Mehmet *et al*. (2008). The high bacterial load in lettuce can be attributed to the large surface area of the leaves suitable for water contact, making them susceptible to bacterial contamination. Ercolani *et al*. (1976) reported an average count for total coliform and faecal coliform of 5.95 x 10^4 and 6.13 x 10^2 cfu/g for lettuce respectively.

The presence of *Enterobacter* spp., *Klebsiella* spp. and *S. aureus* is observed in all samples of salad vegetables in which *S. aureus* was predominant (Table 2). The detection of *S. aureus* is of serious public health importance because of its ability to cause a wide range of infections especially food- borne intoxication (Tambekar *et al*., 2006). Contamination with *S. aureus* has been linked to carriage in nasal passages of food handlers or by infected workers. The presence of *S. aureus* and some Gram negative rods have been reported to contaminate some salad vegetables such as carrots, cucumber, tomato and radishes (Beuchat, 1995).

The presence of *E. coli* in the salad vegetables analyzed is indicative of faecal contamination. *E. coli* are part of the normal flora of the human intestines. Some strains of *E. coli* have been linked to diahorrea, gastro-enteritis and urinary tract infections (Hassan *et al*., 2006).

Klebsiella spp. is second only to *E. coli* as a urinary tract pathogen. It is well known in the environment and can be cultured from soil, water and vegetables when consumed raw as in salads. *E. coli* were also isolated from some leafy green vegetables (Ibrahim, 1996). Khan *et al*. (1992) also isolated *E. coli, Klebsiella* spp. and *Enterobacter* spp. from salad vegetables. In a study done by Tambekar *et al*. (1995), *E. coli* was found to be predominant on some salad vegetables which included coriander followed by carrot, radish, spinach, fenugreek and cucumber.

Salmonella spp. and *Shigella* spp. are non-lactose fermenters usually associated with water contamination. Contamination with these organisms could arise from washing vegetables with contaminated water or handling of vegetables by infected workers. In this study, *Salmonella sp.* was isolated from lettuce. Its presence n food is of serious concern to safety. According to the WHO (2002), effect of microbiological hazards such as *Salmonella* on food safety is now a major public health concern worldwide. Ibrahim (1996) isolated *Salmonella* spp. from lettuce, cucumber and parsley. *Salmonella* spp. has also been isolated from salad vegetables in *waakye* a street food in Ghana. In this study, *Salmonella* spp. was present only in lettuce samples. *Shigella* spp. has been frequently found in salads and dairy products. It is a principal agent of bacterial dysentery.

Pseudomonas spp. is a prominent inhabitant of soil and water. The organism is responsible for diseases of vegetables like angular leaf spot of cucumber (Uzeh *et al*., 2009). Its presence in salad vegetable is also of public health concern as it has been implicated in several infections.

CONCLUSION

In conclusion, the high bacterial load and presence of these organisms especially *E. coli* in the salad vegetable samples could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to poor handling of these vegetables. There is therefore, the need for regulatory bodies to ensure that microbiological standards are established and practiced by farmers and marketers for the handling and distribution of salad vegetables.

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