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BACTERIOLOGICAL QUALITY OF MIXED FRUITS/VEGETABLES SALADS AND SELECTED READY-TO-EAT VEGETABLES SOLD IN ABAKALIKI METROPOLIS, EBONYI STATE, NIGERIA

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ABSTRACT

Food-borne illnesses are major cause of death, worldwide. The range of unhygienic activities surrounding the cultivation, processing and sale of vegetables and vegetable products in Nigeria pose safety challenge on the overall outcome and consumption of vegetables due to the potential for food-borne infections. This study investigated the bacteriological quality of mixed fruits/vegetables salads and some selected ready-to-eat vegetables sold in Abakaliki Metropolis, Ebonyi State, Nigeria. Nine (9) mixed fruits/ vegetables salads purchased from 3 major eateries in Abakaliki, at 3 different sampling days for each eatery were analyzed alongside ready-to-eat leafy vegetables sold at Abakpa market surroundings using standard microbiological techniques. The results revealed high total aerobic plate counts in the range of 1.2×10^6 cfu/g to 4.5×10^6 cfu/g and 1.2×10^8 cfu/g to 5.4×10^8 cfu/g for the ready-to-eat vegetables and mixed fruits/vegetables salad samples respectively. On the other hand, the total coliform counts ranged from 1.3×10^6 cfu/g to 3.1×10^6 cfu/g and 4.5×10^7 cfu/g to 6.1×10^8 cfu/g for the ready-to-eat vegetables and mixed fruits/vegetables salad samples respectively. Fifty (50) bacterial isolates of 7 genera were identified including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp. Those found in the ready-to-eat leafy vegetables included *Staphylococcus aureus* (27.3%), *Escherichia coli* (18.2%), *Salmonella* spp. (18.2%), *Bacillus* spp. (18.2%), *Pseudomonas* spp. (9.09%) and *Klebsiella* spp. (9.09%). Only *S. aureus* (16.7%), *E. coli* (50.0%)and *Klebsiella* spp. (33.3%) were found in the mixed fruits salads sampled while *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp. were found in varying proportions in the mixed vegetable salads sampled. The study revealed that both ready-to-eat vegetables and mixed fruits/vegetables salads sold in Abakaliki metropolis of Ebonyi state Nigeria were contaminated with potential pathogenic bacteria. This is very disturbing as the mixed fruits/vegetables salads were from major eateries in the state capital.

KEYWORDS: Vegetable, ready-to-eat, salad, contamination, bacteria.

INTRODUCTION

The recent years have witnessed an overwhelming interest in the consumption of fresh ready-to-eat fruits and vegetables due to their nutritional and health benefits, and easy availability. Vegetables are rich sources of vitamins, minerals, dietary fibres, important phytochemicals as well as other medicinal and therapeutic agents (Eni *et al.*, 2010; James and Ngarmsak, 2011; Goja and Mahmoud, 2013; Uwamere *et al.* 2013; Odu and Okomuda, 2013). They have been said to decrease the risk of cardiovascular diseases, cancer, obesity, stroke with overall mortality reduction (Adebayo-Tayo *et al.*, 2012; Isa *et al.*, 2014; Elmanama *et al*, 2017). They are either consumed singly or mixed. Salad is a dish of mixed vegetables containing small pieces of vegetables and fruits, often with sauce or salad dressing (Abadias *et al.*, 2008). Understanding and promoting their health benefits, the world health organization (WHO) launched a vegetable promotion initiative that recommends the consumption of a minimum of 400g of fruits and vegetables per day

(WHO, 2003). Therefore, the increasing demand for vegetables has resulted in a wide and uncontrolled vending of vegetables especially in the form of salads.

However, the range of unhygienic activities surrounding the cultivation, processing and sales of vegetables and mixed vegetable salads in Nigeria raises a lot of concern about potential contamination by pathogenic microorganisms. They are frequently exposed to possible contamination at every stage from the farm to the table. Possible sources of contamination of vegetables include soil-borne microbes, fertilization with animal dung, harvesting and transportation equipment, irrigation/washing water, hands of vendor and buyers (Johannessen *et al.,* 2004; Okonko *et al.,* 2008; Adjra *et al.,* 2013; Hannan *et al.,* 2014). Mixed vegetables salads are often taken without further heat treatment in various shops and eateries; hence, outbreaks of food-borne diseases have been associated with the consumption of fresh vegetables in many parts of the world (Levy *et al.,* 2002; Sivapalasingam *et al.,* 2004; Lynch *et al.,* 2009; Elmanama *et al.,* 2017). Previous studies have implicated a number of pathogenic and contaminating bacteria in association with fresh vegetables and fruits used in preparing salads. These include: *S. aureus, Escherichia coli, Enterobacter* spp., *Klebsiella* spp., *Salmonella typhi, Serratia* spp., *Providencia* spp. *Pseudomonas aeruginosa, Yersinia enterocolitica, Campylobacter* spp., *Clostridium botulinum, Bacillus cereus, Listeria monocytogenes Aeromonas hydrophila,* and *Shigella sonnei* (Poorna and Randhir, 2001; Johannessen et al., 2002; Lavelli *et al.,* 2006; Warren et al., 2007; Wright et al., 2009; Xanthopoulos et al., 2009; Adjra *et al.,* 2013). However pathogenic bacteria of human origin may also be present in minimally sanitized vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water insects, animals, workers, harvesting and transportation equipment (Adebayo-Tayo et al., 2012).

In Abakaliki, the capital city of Ebonyi State, Nigeria, fresh vegetables also used for salads are often openly displayed for sale in an unhygienic state on floors, tables, trays, wheelbarrows and handled by intending buyers with bare hands. This condition could result in postharvest contaminations which could influence the overall microbial quality of the vegetables and the salads for which they are used. Also, the non-literate vendors often lack proper personal hygiene and could further contribute to the burden. Since they are often consumed raw without heat treatment, they could pose safety challenges to consumers. We have previously investigated the microbial quality of different ready-to-eat food types vended in Ebonyi state and our study revealed alarming microbial burden (Orji *et al.,* 2016a; Orji *et al.,* 2016b; Orji *et al.,* 2016c). This present study investigated the bacteriological quality of mixed fruits/vegetables salads and ready-to-eat vegetables sold in Abakaliki metropolis, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Abakaliki metropolis of Ebonyi state, south-eastern part of Nigeria. It is bounded by Enugu state by west, Cross River State by the East, Abia State by the south and Benue State by the North and located between longitude 7^0 30^1N and latitude 60^0 45^1E. Abakaliki which is the state capital has a tropical climate with average relative humidity of 75% and may reach 80% during the rainy season. The vegetation characteristics are predominantly rainforest with average atmospheric temperature of about 30^0C. The state enjoys two distinct seasons, rainy season (between April and October) and dry season (November and march) respectively. Ebonyi state according to census (NPC, 2006) has a population of about 2,173,501 people. It is mainly agrarian in nature.

Sample Collection and Processing

In sample collections, three strategic places where chosen around Abakaliki metropolis. They include; Restaurant X, Restaurant Y and Restaurant Z. These restaurants are located along Ogoja road, Water Works road and Izza roads respectively. Mixed fruit salads were purchased from restaurant X, mixed vegetable salad were purchased from restaurant Y and Z respectively. All these purchased samples were purchased between 11 am to 12 noon and transported to Applied Microbiology Laboratory Unit of Ebony State University within 24 hours after collection for Microbiological analysis. The vegetable salad samples from three different food vendors were separated and labelled thus: vendor X, Y, and Z. Then 10g of the samples were homogenized through blending with 90ml peptone water individually in sterile automatic blender and were serially diluted up to 10^{-8} (Cappuccino and Sherman, 1998).

A 0.1ml aliquot of the 10^{-8} dilutions of the different samples was pipette into the petri-dishes and the plates were labelled Vendor X, Y and Z,(Nutrient Agar and Eosin methylene blue Agar) sterilized media were poured onto the samples, swirled gently and left to solidify undisturbed. The plates were labelled as; NA sample X, NA sample Y, NA sample Z, EMB sample X, EMB sample Y, EMB sample Z, and this was done for the different media and different samples. The plates were incubated at 37^0C for 24 hours and the procedure was repeated in duplicate. The two different media (nutrient and eosin methylene blue agar media), already prepared were inoculated with each of the Approximately 0.1ml aliquots of appropriate dilution(10^{-8}), containing samples, the inoculated plates were incubated at 37^0C for 24hrs for growth to occur. After incubation, the inoculated plates were examined for growth and then the morphological characteristics of the isolates were studied (Cheesbrough, 2006).

Identification of Isolates

The two different media (nutrient and eosin methylene blue agar media), already prepared were inoculated with

each of the Approximately 0.1ml aliquots of appropriate dilution (10^{-8}), containing samples, the inoculated plates were incubated at 37^0C for 24hrs for growth to occur. After incubation, the inoculated plates were examined for growth and then the morphological characteristics of the isolates were studied. Purity plates were made for all suspected isolates, after which they were identified using both morphological/culture characteristics and biochemical test (i.e. oxidase, indole, catalase, coagulase test.) for further confirmation of the presence of pathogenic bacteria (Cheesbrough, 2006).

RESULTS

The results of the bacteriological analysis of mixed vegetable/fruits salads and some selected ready-to-eat vegetables samples sold in Abakaliki metropolis for three sampling days revealed high microbial burden. For the different ready-to-eat vegetables (cabbage, lettuce and garden egg), the total aerobic plate counts were within the range of 1.2 x10^6 cfu/g to 4.5 x 10^6 cfu/g, while total coliform counts were within the range 1.3 x 10^6 cfu/g to 3.1 x 10^6 cfu/g. Highest mean counts were recorded by cabbage samples which had mean total aerobic plate counts and total coliform counts of 3.07 x 10^6 cfu/g and 2.9 x 10^6 cfu/g respectively. It also showed that cabbage samples collected from Hausa quarters had the highest bacterial counts. On the other hands, samples of mixed vegetables and fruits salads revealed total aerobic counts within the limits of 1.2 x 10^8 cfu/g to 5.4 x 10^8 cfu/g and total coliforms counts within the limit of 4.5 x 10^7 cfu/g to 6.1 x 10^8 cfu/g (Table 1).

The results on the bacteriological analysis of the samples revealed the presence of 50 bacterial isolates of 7 genera including *Staphylococcus aureus, Escherichia coli, Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp. Those found in the ready-to-eat leafy vegetables included *Staphylococcus aureus* (27.3%), *Escherichia coli* (18.2%), *Salmonella* spp. (18.2%), *Bacillus* spp. (18.2%), *Pseudomonas* spp. (9.09%) and *Klebsiella* spp. (9.09%). Only *S. aureus* (16.7%), *E. coli* (50.0%) and *Klebsiella* spp. (33.3%) were found in the mixed fruits salads sampled while *Staphylococcus aureus, Escherichia coli, Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp. were found in vegetables salads (Table 2).

Table 1: Bacterial load of mixed vegetable salads and selected ready-to-eat vegetables sold in Abakaliki Metropolis (CFU/g).

Sample	Source/Sampling Day	Total Aerobic Plate Count (cfu/g)	Total Coliform Count (cfu/g)
Ready-to-eat vegetables			
Cabbage	Meat Market /Day 1	2.4 x 10^6	2.9 x 10^6
	Abacha Roundabout /Day 1	2.3 x 10^6	2.7 x 10^6
	Housa quarters/ Day 1	4.5 x 10^6	3.1 x 10^6
Mean (cfu/g)		3.07 x 10^6	2.9 x 10^6
Lettuce	Meat market /Day 2	2.0 x 10^6	1.3 x 10^6
	Abacha Roundabout /Day 2	2.0 x 10^6	1.5 x 10^6
	Housa quarters /Day 2	2.2 x 10^6	1.8 x 10^6
Mean (cfu/g)		2.07 x 10^6	1.53 x 10^6
	Meat Market/ Day 3	1.3 x 10^6	2.0 x 10^6
	Abacha Roundabout/ Day 3	1.2 x 10^6	1.6 x 10^6
	Housa quarters/ Day 3	1.6 x 10^6	2.2 x 10^6
Mean (cfu/g)		1.37 x 10^6	1.93 x 10^6
Mixed fruits and vegetable salads			
Fruits salads from X	Eatery X/ Day 1	3.3 X 10^8	3.9 X 10^8
	Eatery X/ Day 2	2.1 X 10^8	1.5 X 10^8
	Eatery X/ Day 3	3.4 X 10^8	6.1 X 10^8
Mean (cfu/g)		2.93 X 10^8	3.83 X 10^8
Vegetables salads from Y	Eatery Y/ Day 1	3.3 X 10^8	4.5 X 10^7
	Eatery Y/ Day 2	5.4 X 10^8	4.5 X 10^8
	Eatery Y/ Day 3	2.7 X 10^8	6.0 X 10^8
Mean (cfu/g)		3.8 X 10^8	3.65 X 10^8
Vegetables salads from Z	Eatery Z/ Day 1	2.4 X 10^8	1.0 X 10^8
	Eatery Z/ Day 2	1.2 X 10^8	1.3 X 10^8
	Eatery Z/ Day 3	1.4 X 10^8	4.2 X 10^8
Mean (cfu/g)		1.67 X 10^8	2.17 X 10^8

Table 2: Occurrence of bacterial isolates in mixed fruits/vegetables salads and selected ready-to-eat vegetables sold in Abakaliki Metropolis (%).

Isolate	Prevalence n(%)				
	Ready-to-eat vegetables	Salads			
		Mixed Fruits salads from X	Vegetables salads from Y	Vegetables salads from Z	Total n(%)
Staphylococcus aureus	9(27.3)	3(16.7)	3(16.67)	2(14.28)	8(16.0)
Escherichia coli	6(18.2)	9(50.0)	3(16.67)	2(14.28)	14(28.0)
Salmonella spp.	6(18.2)	-	-	-	-
Bacillus spp.	6(18.2)	-	-	-	-
Klebsiella spp.	3(9.09)	6(33.3)	6(33.30)	2(14.28)	14(28.0)
Pseudomonas spp.	3(9.09)	-	3(16.67)	4(28.57)	7(14.0)
Serratia spp.	-	-	3(16.67)	4(28.57)	7(14.00)
Total n(%)	**33(100)**	**18(100)**	**18(100)**	**14(100)**	**50(100)**

DISCUSSIONS

The microbial status of ready-to-eat foods and vegetables reflects the sanitary condition of the harvesting, processing and marketing environment as well as the personal hygiene of the handlers. The bacteriological analysis of the ready-to-eat vegetables and salads sold in Abakaliki metropolis revealed a very low microbial quality. The total aerobic and coliform counts per gram of samples in this study were higher in the salads than the ready-to-eat vegetables. The high microbial burden recorded in this study is in line with previous studies from Bangladesh (Kundu and Islam, 2015), Togo (Adjrah *et al.*, 2013), Iran (Najafi and Bahreini, 2012), and Hong Kong (HKSAR, 2002) reported almost similar data which coincide with our study. Also, Hannan *et al.* (2014) reported a microbial load of up to 5.8 x 10^8 cfu/g of dry vegetable salads in Lahore, Pakistan. However, it does not corroborate with the reports of Uwamere *et al.* (2013) who recorded highest total heterophilic counts of 2.8 x 10^4 cfu/g for vegetable salads in Edo State, Nigeria. Also, Wogu and Iwezeua (2013) reported a total colony count in the range of 3. 01 x 10^1 cfu/g to 1.5 x 10^3 cfu/g on ready-to-eat vegetable salads sold in Benin City, Nigeria. The variation in results recorded could be due to differences in the sanitary conditions of the study areas, the personal hygiene of the handlers and the nature of the vegetables studied. The high total aerobic and total coliform counts could have resulted from the interplay of many factors such as poor personal hygiene of handlers, exposure to dust, use of animal faeces for fertilization of the soil on which these vegetables were grown, use of contaminated water for irrigation/washing, faecal contamination, handlers who often touch the vegetables with bare hands in trying to choose which to buy (Johannessen *et al.*, 2004; Okonko *et al.*, 2008; Adjra *et al.*, 2013; Hannan *et al.*, 2014). Halablab *et al.* (2011) noted that total coliform counts could be considered as a hygiene indicator especially for fecal contamination. Also, the higher counts observed in the salads than the ready-to-eat vegetables is not surprising as the preparation of mixed vegetable salads often require exposing the vegetables to all manner of cutting which increases the surface area and potential for microbial colonization. Also, Garg *et al.* (1990) and Farmer (1995) previously noted that exposing vegetables to various types of cutting has been shown to result in a six to seven-fold increase in microbial numbers.

Furthermore, the study revealed the presence of seven (7) bacterial genera associated with ready-to-eat vegetables and fruits/vegetable salads and these included *Staphylococcus aureus, Escherichia coli, Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp. All the bacteria reported in this study had previously been isolated from vegetables in other studies both in Nigeria and elsewhere (Dunn et al., 1995; Uwamere *et al.*, 2013; Wogu and Iwezeua, 2013; Sabbithi *et al.*, 2014; Oliveira *et al.*, 2015; Coniglio *et al.*, 2016). Halablab *et al.* (2011) reported that lettuce samples from Bekaa Valley in Lebanon had *E. coli, S. aureus* and coliforms. In the report of Rajvanshi (2010), all samples of ready-to-eat vegetables in Jaipur city, carried gram positive and gram negative bacteria including *Bacillus* (24.5%), *E. coli* (11.8%), *Pseudomonas* species (11.8%), *Staphylococcus* species (10.9%) and *Klebsiella* species (5.4%). However, our study revealed percentage occurrences of the various isolates which were higher or lower than other previous reports.

The isolation of *E. coli, Klebsiella* spp., *Serratia* spp. and *Salmonella* spp. coupled with high coliform counts recorded in this study is very alarmingly disturbing as it suggests gross faecal contamination of the sampled mixed fruits and vegetable salads. This raises a concern about the personal hygiene of the vendors as well as the sanitary condition of the processing environment. Also, the use of animal dung in growing this vegetables could have contributed to the high presence of faecal organisms (Olayemi, 2007; Halablab *et al.*, 2011; Kundu and Islam, 2015). This finding supports other previous studies of Eni *et al.* (2010) in Ota, Nigeria and Coniglio *et al.* (2016), who also isolated both organisms from fruits and vegetables. However, our study recorded higher occurrence rates.

The high levels of *S. aureus* in most of the salads sold in the restaurants studies indicate poor handling practices

during and/or after salad preparation. The common sources of *S. aureus* in food are the nose, throat, skin, and hair of healthy humans and animals as well as feathers of birds where they occur naturally (Garvani, 2002). Food handlers are the main agents of transmission of *S. aureus* into food. According to Loir *et al*. (2003), improper handling of food by contaminated hands or other improper food handling practices such as coughing or sneezing during food preparation usually after heat treatment of the food contribute significantly to *S. aureus* contamination of food. FDA (2012) also indicated that, unless heat processes are applied, staphylococci are expected to exist in any as well as all foods that are handled directly by humans or are of animal origin. *S. aureus* species are mainly involved in staphylococcal food intoxication cases (Khambaty *et al.*, 2004). According to FDA (2012), the intoxication dose of staphylococcal enterotoxins (SE) is less than 1.0µg which can be produced when *S. aureus* populations exceed 100, 000 organisms/g in food. Sufficient moisture, ambient temperature and adequate time will ensure a continuing increase in the bacteria population (Adebayo-Tayo *et al.*, 2012). The occurrence of *Bacillus* spp. could have resulted from exposure to dust and air as the spores of the organism are often carried in the air and can germinate on food given adequate conditions of growth.

CONCLUSION

The present study clearly demonstrated that the vegetable salads and ready-to-eat vegetables sold in Abakaliki metropolis of Ebonyi State, Nigeria were highly contaminated with potential pathogenic bacteria. The presence of enteric bacteria such as *Escherichi coli*, *Klebsiella* species, *Pseudomonas* species and *Serattia* species in the salad samples may be suggestive of faecal contamination due to poor hygiene and sanitation. This may play a significant role in the transmission of various diseases. Environmental sanitation, proper handling and personal hygiene are required to prevent contamination of vegetables and their mixed salad products.

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