



## Assessment of pathogenic microorganisms associated with vegetable salads sold in otuoke business district

Opara C N<sup>1</sup>, Okoronkwo K A<sup>2</sup>

<sup>1</sup> Department of Microbiology, Faculty of Science, Federal University, Otuoke, Bayelsa State, Nigeria

<sup>2</sup> Department of Biological Sciences (Microbiology Programme), Faculty of Science, Edwin Clark University, Kiagbodo, Delta State, Nigeria

### Abstract

Food-borne diseases are global public health problem. These play a significant role in human morbidity, mortality and economic loss. Vegetables salad are considered as an 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. The study was performed to determine pathogenic microorganism associated with vegetable salad sold in Otuoke of Bayelsa State, Nigeria. Samples of vegetable salad were collected from different food vendors in Otuoke and were evaluated for bacterial loads and sensitivity test. Bacterial loads ranged from  $1.6 \times 10^6$  to  $2.9 \times 10^8$  cfu/g. *E.coli*, *Staphylococcus*, *Enterobacter* spp., *Klebsiella* spp. and *S. aureus* were associated with the samples. *Staphylococcus*, *E.coli* and *S. aureus* was isolated from majority of the samples. Transmission of resistant bacteria is a potential concern with unhygienic handling of vegetable salads. Better handling process and health education is crucial to preventing spread of resistant bacteria and food poisoning through these otherwise commonly consumed vegetable salads.

**Keywords:** pathogenic microorganisms, business district, *Staphylococcus*, *Enterobacter*

### Introduction

Food safety issues have been given much attention in recent times because of increasing food related illnesses (Peattie, 2016; Leech, 2015) [13, 11]. According to WHO (2015), food safety measures must be in place during production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, wholesome and fit for human consumption. Food safety, thus, involves everyone in the food supply system, from production to processing and consumption. Governments, law makers, food manufacturers, caterers, food vendors, farmers, and all consumers have roles to play in making food safe (Ababio and Adi, 2012) [1].

Consumption of fresh fruits and vegetables is encouraged in recent years. According to James and Ngarmak (2017) [9], Salad vegetables are usually not subjected to any form of heat treatment or may be partially cooked before consumption. Additionally, adequate washing and peeling may not be employed in extreme cases making consumption of the commodity a potential vehicle for food borne diseases (Tambekar and Mundhada, 2016) [15].

Pathogenic bacteria can contaminate vegetables at any stage from planting to consumption. The use of untreated wastewater and water supplies contaminated with sewage used for irrigation, post-harvest handling, and preparation in unhygienic environments in food services and home settings are among the commonly reported sources of vegetable contamination (Amoah *et al.*, 2017) [3]. *Staphylococcus aureus*, *Enterobacter* spp., *Salmonella typhi*, *Pseudomonas aeruginosa* and *Shigella sonnei* are among the commonly isolated pathogens from vegetable salads (Poorna and Randhir, 2016) [14]. Microbial contamination of vegetables can have negative effect on the product including spoilage, decreased sensory appeal and decreased shelf life. According to Halablab *et al.*, (2016) [7], outbreaks of

foodborne illnesses related to vegetable consumption can be large or small, ranging from few numbers of persons to thousands. For example, Meldrum *et al.*, (2019) [12] reported two large outbreaks in the United Kingdom which were attributed to the consumption of contaminated vegetable salads. A study by Amoah *et al.*, (2017) [3], indicated that fresh vegetables salad has become a normal part of fast food served on the street, canteens and restaurants in Nigeria.

(Khan *et al.* 2012) [10] reported that bacterial contamination results from various unsanitary cultivation and marketing practices. In another study, (Tambekar *et al.* 2016) [15] 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) [8]. This study therefore investigates the pathogenic microorganisms associated with salads.

### Materials and methods

#### Study area

The study was conducted in Otuoke Business District which is the heart of the Ogbia Metropolis of Bayelsa state where most business activities take place. It is specifically regarded as the fastest-growing city in goods from the agricultural and commerce sectors from other districts in the region (Otuoke Region area in Bayelsa state).

### Sample Collection and Processing

The study area was stratified into six different food vendor in Otuoke, all in the Central Business District in Otuoke metropolis (CBD considered in this study composed of cabbage, six samples of ready-to-eat (pre-cut salads for ready consumption, locally, made based on the number of vendors of vegetable salads in each group one (1) one samples were taken from 1, one samples of vegetable salad mixtures from each vendor which are usually served directly to consumers were aseptically collected into sterile polythene zip lock bags, kept in ice chest, maintained at 0–4 °C and were then transported to the Laboratory of the University for microbiological analysis within 2–4 h for microbial analysis.

### Media Preparation

The media used for the study are Nutrient Agar, MacConkey Agar (mCA), Salmonella-Shigella agar (SSA), and Potato Dextrose Agar (PDA) used to isolate fungi. Each medium was accordingly weighed and dissolved in appropriate quality distilled water. The Agar media are prepared mainly based on the manufacturer's instructions.

### Preparation of Salad Samples

Twenty-five grams (25 g) of Salad was mixed thoroughly with 225 ml of buffered peptone water. This mixture was

homogenized very well by simple “hand massaging” and constant shaking to obtain a uniform mixture (stock). Ten (10)-fold serial dilutions were also carried out at five (5) levels.

### Bacteria Isolation and Identification

After 24 hours of incubation, predominant bacteria colonies were sub-cultured using already prepared MacConkey agar and nutrient agar plates. Sterilized platinum inoculation loops (Thomas Scientific, New Jersey, USA) were used to streak isolated bacteria on already prepared MacConkey agar and Nutrient Agar respectively to obtain pure bacterial sub-cultures, after which they were incubated at 37°C for 24 hours. Identities of bacteria cultures were confirmed using morphological characteristics, and gram positive and gram negative standard biochemical tests (Cheesbrough, 2006) [15].

### Statistical Analysis

Experiments were carried out in triplicates. The data obtained were analyzed by one-way analysis of variance (ANOVA) and the means compared by New Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at  $p < 0.05$ .

### Results and discussion

**Table 1:** Total viable count of Salad Vegetable

No of sampling	Sampling site	Type of Sampling	Total Viable Count (cfu/g)
1	Otuoke	Salad	$2.9 \times 10^8$ SD $7.20 \times 10^1$
2	Otuoke	Salad	$1.6 \times 10^8$ SD $5.70 \times 10^1$
3	Otuoke	Salad	$1.6 \times 10^6$ SD $6.50 \times 10^8$
4	Otuoke	Salad	$1.9 \times 10^8$ SD $1.70 \times 10^7$
5	Otuoke	Salad	$4.6 \times 10^7$ SD $2.83 \times 10^6$
6	Otuoke	Salad	$2.4 \times 10^7$ SD $1.70 \times 10^7$

Key: SD = Standard deviation

The total viable count was done on MacConkey agar (Oxoid, England) by spread plate method. Among the various samples of vegetable salad analyzed, salad 2, samples sourced from Otuoke food vendor had the highest total viable count of  $1.6 \times 10^8$  cfu/g. This was higher than the total viable count of  $5.7 \times 10^6$  cfu/g for salad vegetable salad samples reported by Uzeh *et al.* (2009) [16]. Rheinlander (2016) suggested that a limit of 10 cfu/g should be standard with market raw food. This finding implies that since vegetable used for salad such as 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 salad 3 samples was gotten from samples sourced from Otuoke with a load of  $2.4 \times 10^7$  cfu/g while those sourced from same Otuoke had the lowest load of  $1.6 \times 10^6$  cfu/g. This result was comparable to the bacterial load of  $1.9 \times 10^6$  cfu/g reported by Abdullahi *et al.* (2010) [2]. Unlike salad 2, salad 3 are rarely contaminated. Contamination with these pathogens could be due to poor hygiene practices by handlers. Salad 4 samples sourced from Otuoke had the highest coliform load of  $2.9 \times 10^8$  cfu/g. This was higher than a load of  $6.9 \times 10^6$  cfu/g reported for salad 4 samples. The high bacterial load in salad 4 can be attributed to the large surface area of the leaves suitable for water contact, making them susceptible to bacterial contamination. This finding implies that since vegetable used for salad such as

carrots are usually harvested from the soil hence can become contaminated by pathogenic organisms in soil. Among the three different salad vegetables analyzed, salad samples 3 had the lowest bacterial load. The highest total viable count for salad 3 samples was gotten from samples sourced from Otuoke with a load of  $2.4 \times 10^7$  cfu/g while those sourced from same Otuoke had the lowest load of  $1.6 \times 10^6$  cfu/g. This result was comparable to the bacterial load of  $1.9 \times 10^6$  cfu/g reported by Abdullahi *et al.* (2010) [2]. Unlike salad 2, salad 3 are rarely contaminated. Contamination with these pathogens could be due to poor hygiene practices by handlers. Salad 4 samples sourced from Otuoke had the highest coliform load of  $2.9 \times 10^8$  cfu/g. This was higher than a load of  $6.9 \times 10^6$  cfu/g reported for salad 4 samples by Mehmet *et al.* (2008). The high bacterial load in salad 4 can be attributed to the large surface area of the leaves suitable for water contact, making them susceptible to bacterial contamination. The biochemical test indicates the presence of *E.coli*, *Staphylococcus spp*, *Enterobacter spp.*, and *Klebsiella spp* observed in all samples of salad vegetables. 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.*, 2016) [15]. 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, 2015). 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 diarrhoea, gastro-enteritis and urinary

tract infections (Hassan *et al.*,2016). *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.

**Table 2:** Results of biochemical tests of the pathogenic isolates

	Motility	Oxidase	Catalase	Citrate	Grams staining	Organisms
1	-	+	+	+	+	<i>Staphylococcus</i>
2	-	-	+	-	-	<i>E. coli</i>
3	+	+	+	+		<i>Klebsiella</i> spp
4	-	+	+	-		<i>S. aureus</i>
5	+	+	+	+		<i>Enterobacter</i> spp

+ = Positive, - = Negative

**Table 3:** Antimicrobial Sensitivity Test for Gram positive and negative Bacteria Isolates from vegetable salads

Antibiotics	<i>E.coli</i>	<i>Staph</i>	<i>Shigella</i>	<i>Enterobacter</i>	Interpretation
Seprtrin	30	34	20	32	sensitive
Streptomycin	32	32	30	36	
Ampicillin	---	---	18	34	
Ceporex	36	12	34	--	
Ofloxacin	36	30	36	36	
Nalidilicacid	30	28	18	12	
Pefloxacin	32	30	39	36	
Gentamycin	34	32	34	30	
Augmentin	38	36	36	34	
Ciprofloxacin	32	30	36	36	

-: No sensitivity.

The result of sensitivity test performed on Gram positive and negative bacteria isolated from the various samples of vegetable salad are shown in table 3. All the gram positive isolate were + sensitive to Seprtrin, Streptomycin, Ofloxacin, Nalidilic acid, Pefloxacin, Gentamycin, Augmentin and Ciprofloxacin. These bacterial isolate sensitive to the antibiotics were *E.coli*, *Staph*, *Shigella* and *Enterobacter*. They were intermediate to sensitive to all the antibiotics except Ampicillin. *E.coli* were intermediate to sensitive to all the antibiotics tested. The result of the anti-microbial sensitivity test is presented. Many of the bacteria show an intermediate to sensitive reaction to most of the antibiotics tested. All the gram positive isolate were + sensitive to Seprtrin, Streptomycin, Ofloxacin, Nalidilic acid, Pefloxacin, Gentamycin, Augmentin and Ciprofloxacin. These bacteria isolate sensitive to the antibiotics were *E.coli*, *Staph*, *Shigella* and *Enterobacter*. They were intermediate to sensitive to all the antibiotics except Ampicillin; *E.coli* were intermediate to sensitive to all the antibiotics tested. These findings is supported by the works of Daniyan and Ajibo (2011) [6] who reported that *S.aureus*, *E.coli*, *Staph*, *Shigella* and *Enterobacter* were resistant to pefloxacin but susceptible to Streptomycin, Ciprofloxacin, Ceftriaxone and Cefuroxime. Srinu *et al* (2012) also reported that *S.aureus*, *E.coli*, *Staph*, *Shigella* and *Enterobacter* were sensitive to streptomycin, cotrimoxazole and Ciprofloxacin. Marwa *et al* (2012) reported that most *E.coli* isolates from food were sensitive to Amoxicillin /Clavulanic acid, Cefuroxime, Ciprofloxacin, Ofloxacin, Sparfloxacin and Cotrimoxazole. Srinu *et al* (2012) also reported that *E.coli* was sensitive to Streptomycin, Cotrimoxazole and ciprofloxacin.

**Table 4:** Fungi Isolated from vegetable salads

Sample plates	Isolated Organisms	Total
1	<i>Rhizostonia solani</i>	19(24.1%)
2	<i>Microphomina phaseolina aspergillus vsicolor</i>	17(21.5%)
3	<i>Rhizostonia solani</i>	9(11.4%)
4	<i>Microphomina phaseolina aspergillus vsicolor</i>	8(10.1%)
5	<i>Microphomina phaseolina aspergillus vsicolor</i>	7(8.9%)
	Total	32(100.0)

The result of fungi isolated from the various sample of vegetable salad. It was revealed that *Rhizostonia solani* was present in sample 1 and 3 (24.1% and 11.4%),), while *Microphomina phaseolina aspergillus vsicolor* was present in sample 2, 4 and 5 (21.5%),(8.9%) and (10.1%).The isolated fungi from the vegetable salad showed that the highest presence of *Rhizostonia solani* could be due to its pharmacokinetic properties. However, it is a serious threat to public health as contaminated salads harbouring the antibiotic resistant microorganisms end up in the food without treatment.

**Conclusion**

The pathogenic microorganism associated with salads as indicated in the laboratory result and analysis shows that the high bacterial load and presence of these pathogenic microorganisms especially *E.coli* and *Staphylococcus* in the vegetable salad 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.

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