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## Microbial Contamination of Fresh Vegetable Salads from Food Vendors in Oyo Metropolis

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### ABSTRACT

Vegetables provide humans with essential vitamins and minerals with enough ability to help reduce the risk of diseases and infections. However, a major issue associated with the consumption of fresh vegetable salad is their contamination with pathogenic microorganisms. Thus, this study was aimed at assessing the microbial contamination levels and evaluating the microbiological risk associated with the consumption of ready to eat mixed vegetable salads from food vendors in Oyo, Oyo state. Microbiological analysis was done using standard methods. Results showed that from 20 samples, a total of fifty-five (55) isolates were gotten, while 91% of the isolates were bacteria, 9% were fungi. The total bacteria count obtained in this study ranged from  $6.00 \times 10^5$  to  $7.30 \times 10^5$  cfu/ml. The bacterial isolates identified from the samples were: *Aeromonas* sp. (50%); *Serratia* sp. (20%); *Pseudomonas* sp. (21%); *Escherichia coli* (3%); *Lactobacillus* sp. (3%); and *Staphylococcus* sp. (3%). The fungal count realized in this study ranged from  $1.67 \times 10^4$  cfu/ml to  $2.90 \times 10^4$  cfu/ml. The fungal isolates were: *Aspergillus* sp.; and *Rhizopus* sp.; (78%) and (22%) respectively. The high microbial load found in the vegetable salad samples shows that there is an urgent need for food handlers to employ appropriate decontamination methods. Consumed food being sold by vendors must always meet up with stipulated microbiological standards for consumed food.

**Keywords: Contaminants, Vegetables, Food, Microbial**

### Introduction

Vegetables remain a crucial part of a healthy diet, making the demand for fresh vegetable salads constantly on the increase. Vegetables provide humans with essential vitamins and minerals with

enough ability to help reduce the risk of diseases and infections (Sharma and Sekhon, 2016). The World Health Organization (WHO) and Food and Agricultural Organization (FAO) recommend regular intake of vegetables (Toe *et al.*, 2017). The

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health benefits of salad have increased its use for the purposes of weight loss, medical disorders and strokes.

Page | 3375 A foremost issue associated with the consumption of fresh vegetable salad is their contamination with pathogenic microorganisms (Feng, 2002). Vegetable salads are consumed uncooked, with washing being the major means of removing contaminants and microorganisms. This method of decontamination is not often sufficient to remove all pathogenic organisms from the vegetables. Various factors account for the high risk of microbial contamination associated with vegetable salads. This ranges from environmental conditions (hygiene) where these vegetable salads are prepared, washing of the vegetables using contaminated water, cultivation, post-harvest handling to transportation (Taban and Halkman, 2011). Other ingredients used in the making of the vegetable salad could also be a source of contaminant (Gantois *et al.*, 2009). Thus, from planting, harvest to processing and consumption, the probability of these vegetable salads coming in contact with pathogenic microorganisms is very high.

The most common organisms linked with contamination of vegetable salads include; *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, *E. coli*, *Staphylococcus aureus*, *Shigella sonnei*, *Salmonella typhi*, *Pseudomonas aeruginosa* (De Rover, 1998). Studies have shown that these organisms are among the most common

foodborne pathogens in Nigeria (Nkere *et al.*, 2011).

In Nigeria, vegetables and raw materials for the preparation of vegetable salads are often sold in open unhygienic places. The major means of removing pathogens remains washing and often times the sources of water are also contaminated. The vendors of these vegetable salads often sell them in open places which are often not germ-free. Thus, this study was to assess the microbial contamination levels and evaluate the microbiological risk associated with the consumption of ready to eat mixed vegetable salads from food vendors in Oyo, Oyo state.

## **Methodology**

### ***Sample collection and processing***

Twenty samples were collected from twenty different vendors around Oyo metropolis. The vendors were randomly selective using convenience sampling. The samples were collected exactly as they were displayed for sale in vendor and put in sterile polyethylene bags and taken directly to the laboratory for analysis.

### ***Microbiological analysis***

Microbiological analysis was done using standard methods. Aerobic plate count (APC) was performed by the pour plate method using plate count agar (PCA). Stock solution of  $10^{-1}$  dilution was prepared by homogenizing 5g of the sample in 9ml of sterile water. Serial dilutions of  $10^{-1}$  to  $10^{-6}$  were prepared by tenfold dilution of each preceding dilution. A 1 ml aliquot from each

dilution was poured onto the centre of Petri dishes and sterile molten PCA poured into the Petri dishes already containing the samples. The culture and the medium were mixed thoroughly by gently moving the plates and the plates were allowed to solidify and incubated at 37°C for 24 hours in an inverted position. After 24-hour incubation, colonies on PCA were counted and recorded in CFU/g of salad samples. For determination of yeasts and molds, 1 mL of each decimal dilution was placed on plate surface that contained Potatoes dextrose agar (PDA) and distributed by a sterilized swab. Plates were incubated for 3 days at 25°C room temperature.

#### **Characterization and Identification of bacteria isolates**

The characterization and identification of bacteria isolates were based on their colonial morphology and biochemical characteristics. The colonial morphology of the microorganisms was based on the shapes, sizes, optical character, consistency, elevation and pigmentation while the cellular morphology was determined through microscopic examination and staining techniques. After characterization, tentative identification was done using Bergey's Manual of Determinative Bacteriology. Standard biochemical test ranging from catalase test, coagulase test, indole test, sugar fermentation test and hydrogen sulphide production.

#### **Identification of Fungi isolates**

The fungal isolates were sub-cultured by transferring a piece of each typical colony from its

edge on to a sterile fresh media and incubated at room temperature for 3-5 days. Morphological observations were based on colour and textures. The spreading rate of each colony on the potato dextrose agar plate and the microscope studies of each type were done for further identification.

Identification of fungi isolate was carried out by adding a drop of lacto-phenol cotton blue stain on a clean grease free slide. A sterile inoculating needle was used to tease out a fragment of the fungi and then transferred into the stain on the slide. This was covered with a cover slip before been examined under low power objective of the microscope  $\times 40$ .

#### **Results**

Results showed that from the 20 samples, a total of fifty-five (55) isolates were gotten from the sampling trips, while 91% of the isolates were bacteria, 9% were fungi. The total bacteria count obtained in this study ranged from  $6.00 \times 10^5$  to  $7.30 \times 10^5$  cfu/ml. The bacterial isolates identified from the samples were: *Aeromonas* sp. (50%); *Serratia* sp. (20%); *Pseudomonas* sp. (21%); *Escherichia coli* (3%); *Lactobacillus* sp. (3%); and *Staphylococcus* sp. (3%). The fungal count realized in this study ranged from  $1.67 \times 10^4$  cfu/ml to  $2.90 \times 10^4$  cfu/ml. The fungal isolates were: *Aspergillus* sp.; and *Rhizopus* sp.; (78%) and (22%) respectively as shown in Table 1. Table 2 shows the prevalence of fungi in the study.

Table 1: shows the prevalence of bacteria in each of the food vendors with *Aeromonas* species 35%

| S/N | ISOLATED BACTERIA              | NUMBER OF OCCURENCE | FREQUENCY OF OCCURRENCE (%) |
|-----|--------------------------------|---------------------|-----------------------------|
| 1   | <i>Aeromonas</i> species       | 17                  | 39.53                       |
| 2   | <i>Serratia</i> species        | 7                   | 16.3                        |
| 3   | <i>Pseudomonas</i> species     | 7                   | 16.3                        |
| 4   | <i>Corynebacterium</i> species | 5                   | 11.63                       |
| 5   | <i>Klebsiella</i> species      | 4                   | 9.3                         |
| 6   | <i>Escherichia coli</i>        | 1                   | 2.3                         |
| 7   | <i>Lactobacillus</i> species   | 1                   | 2.3                         |
| 8   | <i>Staphylococcus</i> species  | 1                   | 2.3                         |

Table 2: The prevalence of fungi isolated from vegetable salads

| S/N | ISOLATED FUNGI             | NUMBER OF OCCURRENCE | FREQUENCY OF OCCURRENCE (%) |
|-----|----------------------------|----------------------|-----------------------------|
| 1   | <i>Aspergillus</i> species | 7                    | 77                          |
| 2   | <i>Rhizopus</i> species    | 3                    | 22                          |

**Discussion**

The microbial load present in packaged food is a major factor in ascertaining the sanitary condition of the food. High microbial load in food especially vegetables that are consumed raw or with minimal cooking poses serious health challenges to the consumer. Results from this study showed that the vegetable salads were contaminated and might not be safe for consumption. The high microbial load

in some of the vegetable salad sample is not surprising as the major control mechanism against pathogens is thorough washing and use of food grade chemicals to kill the microorganisms. Similar results were obtained by Odebisi-Omokanye *et al.* (2015); Oranusi and Olurunfemi, (2011).

In this study, pathogenic organisms such as *Escherichia coli* were isolated, which has been reported to be a common cause of outbreak of food borne diseases. Enteric pathogens are among the human pathogens to pose the greatest risks when present in fresh vegetables (Halablab *et al.*, 2011). Enteric pathogens potentials to breed in salads prior to consumption and when combined with their low infective dose, increases the risk of an outbreak. In this study the potential pathogens isolated were *Aeromonas* sp. *Serratia* spp. *Pseudomonas* spp. *Escherichia coli*, *Lactobacillus* sp. and *Streptococcus* sp. Which corroborates the findings of Tony *et al.* (2003).

The high incidence of bacterial contamination of ready to eat salads reported in this study may partly be accounted for by the large numbers of people (usually handlers and sales persons) deployed to assist in the preparation of vegetable salads, who may not observe basic sanitation requirements for processing produce that require no pre-heating before consumption. Furthermore, contamination may occur during the cultivation process as the use of animal manure or irrigation with water contaminated by faecal matter can help introduce pathogens into these vegetables.

Furthermore, cut surfaces (common with vegetables), increases the risk of contamination with microbes as it allows microbial infiltration of the tissues (Garg *et al.*, 1990). The presence of these coliforms especially *E. coli* could also be attributed to human and animal faecal contamination of the vegetable salads along the production processes or during cultivation. These findings have remarkable similarities with the report of El Gohany (1994) who stated that coliforms are a major contaminant of food materials.

A major significance of this study was the isolation of fungal species from the vegetable salad samples. *Aspergillus* sp. were found to have occurred more than *Rhizopus* sp. Various factors have been known to help introduce fungi into vegetables. These include; irrigation water, soil, organic manure, harvesting equipment as well as improper handling during the salad production processes.

Storage conditions of mixed vegetable salads during sales and the frequency of consumption, respectively represent the post processing handling practices and consumption patterns that contribute significantly to the microbiological quality of mixed vegetable salads from canteens in Oyo and their risk of microbial infection.

## Conclusion

The high microbial load found in the vegetable salad samples shows that there is an urgent need for food handlers to employ appropriate decontamination methods. Consumed food being

sold by vendors must always meet up with microbiological standards for consumed food.

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