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Comparative Bacteriological Analysis of Ready-to-Eat Vegetables Salad Sold by Various Food Vendors in Awka

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Abstract: Microbiological quality of mixed ready-to-eat vegetable salads obtained from various sources was conducted to evaluate the level of microbial contamination of different vegetable salad sold through different outlets in Awka, Anambra state. A total of eighty two (82) samples including 40 samples obtained from street-vendors, 40 samples obtained from fast-food joints and 2 laboratory prepared salad samples were analysed. Standard microbiological procedures were used to determine the total aerobic mesophilic bacterial count, total coliform count, total fecal coliform count and the presence of pathogenic bacteria of the samples. The highest aerobic mesophilic bacterial counts were obtained from street vendors samples (5.16 to 6.75log10 CFU/g and 6.51 to 7.66log10 CFU/g) for morning and afternoon samples respectively. The samples from fast-food joints had significantly lower microbial count than the street vended samples (4.15 to 4.74log10 CFU/g and 4.69 to 4.98log10CFU/g) for morning and afternoon samples respectively. The least count was found in the laboratory prepared salad samples (3.50log10 CFU/g and 3.54log10CFU/g) for morning and afternoon counts respectively. Similar observations were made in the total coliform count and total fecal coliform count. Microorganisms isolated from the samples include Escherichia coli (85.36%), Salmonella (26.82%), Shigella (30.48%), Staphylococcus (70.73%), Serratia (47.56%), Streptococcus (78.04%), Micrococcus (63.41%), Pseudomonas (40.24%) and Klebsiella (69.51%). The study revealed the presence of potentially pathogenic microorganisms in the vegetable salad especially in samples from street vendors.

Keywords: Microbiological Analysis, Salad Vegetables, Food Vendors.

INTRODUCTION

Vegetable salad is a term broadly applied to many food preparations that have a mixture of chopped or sliced ingredients of vegetables and salad cream[1]. The vegetables that usually make up this recipe include tomatoes, cucumber, carrot, green beans, cabbage, lettuce, onion, green pepper, among others. This vegetable salad is a common food in Nigeria and the world at large [2]. Vegetable salad can be a vehicle for the transmission of pathogens capable of causing human illness. Tambetar and Mundhada[3] reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any pre-heat treatment. The vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling,

harvesting equipment, transport containers, wild and domestic animals and food handlers during preparation of vegetable salad in fast-food joints and by the street vendors [4].

The incidence of food borne disease outbreaks caused by contaminated fresh vegetables and fruits has increased in recent years [5]. Salmonella and E.coli 0157:H7 outbreak affected 26 USA states which involved about 200 cases of illness, including some hemolytic uremic syndrome (HUS) and resulted in three deaths [6]. The distribution and supply of raw salad vegetables by marketers around the world have expanded the geographical distribution and incidence of human illness associated with consumption of microbial contaminated vegetable salads[7]. Owing to lack of food borne diseases investigations and surveillance in most developing countries, most food borne disease outbreaks go undetected and cause a large proportion of illness[6].

The main objective of this study, therefore, is to investigate and compare the microbiological quality of vegetable salad obtained from fast- food joints, street-vended and laboratory-prepared ready-to-eat vegetable salad.

MATERIAL AND METHODS

Sample Collection

A total of 80 mixed vegetable salad samples were obtained routinely at different time of the day (morning and afternoon) from ten fast-food joints and ten street vendors. The samples were collected aseptically in sterile ice polythene bags and conveyed to the laboratory for microbiological analysis. Two different samples were prepared in the laboratory, making a total of 82 samples examined.

The laboratory prepared vegetable salad, fast foods vegetable salad and street vended vegetable salad were composed of the following ingredients; carrot, cabbage, green beans, onion, tomatoes and green pepper.

Representative portions of each of the vegetable salad were collected and macerated aseptically and 20grammes of each macerated sample was then mixed together to obtain the laboratory vegetable salad.

Determination of Total Aerobic Mesophilic Bacterial Count

Total Aerobic Mesophilic Bacterial Count was done by pour plate technique. 1ml each of 10^{-1} to 10^{-6} dilutions were transferred aseptically into sterile petri-dishes arranged in triplicates using sterile pipettes. The aliquots in the plates were then flooded with 15- 20ml of sterile nutrient agar containing 50mg/100ml nystatin to suppress the growth of fungi. The plates were rocked to ensure even distribution of the inoculums. The plates were allowed to solidify and then were incubated for 24h at 37˚C. Colonies that developed were thereafter counted and expressed as colony forming unit per gramme (CFU/g) [8].

Determination of Total Coliform Count

Total coliform counts of the salad samples were determined by direct plate count method as described by Mehmet and Aydin[7]. Direct plate count was done using MacConkey Agar. Tenfold serial dilutions of the samples were made in sterile distilled water. 1ml of each of the dilution (10^{-1} to 10^{-5}) was introduced and spread on MacConkey agar in triplicates. The plates were incubated for 48h at 37˚C. Pinkish colonies indicating lactose fermenters were counted.

Determination of Faecal Coliform

Total faecal coliform count of all the samples were determined by plate count as described by Mehmet and Aydin[7] using Eosin methylene blue agar. Serial dilutions of the samples were made in sterile distilled water. 1ml of each dilution was plated out using pour plate on Eosin methylene blue agar in triplicate. Colonies having green metallic sheen were counted as faecal coliforms[9].

Isolation and detection of pathogenic bacteria

Standard enrichment and selective culture procedures[10; 11] were used to determine the presence of bacteria in the salad samples.

Pure cultures of the isolates were identified following the methods described by Cheesbrough[12]. Tests carried out include Gram reaction, catalase test, coagulase test, urease test, starch hydrolysis test, citrate utilization test, motility test, methyl red, Voges-Proskauer test, and sugar fermentation test.

RESULTS

The aerobic mesophilic bacterial count, total coliform count and fecal coliform count results were expressed as Logarithmic value of mean plus or minus standard deviation of the triplicate measurements. Out of the eighty-two salad samples examined for bacterial contamination, ten genera were isolated, namely, Pseudomonas, Serratia, Escherichia, Salmonella, Shigella, Bacillus, Proteus, Micrococcus, Klebsiella, Streptococcus and Staphylococcus. The isolation of these organisms from vegetable salads was also reported by Aboh et al., [2] and Ankita[4]. Aboh ET al., [2] isolated Proteus spp, E.coli, Enterobacter spp, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp., Shigella spp., Klebsiella spp. and reported that these organisms are common pathogens found on vegetables. However, Ankita[4] also isolated Citrobacter, Bacillus, and coli, Pseudomonas, Enterobacter, Klebsiella, Lactobacillus and Leuconostoc in his study of the bacterial load on street vended salads. The frequency and percentage occurrence of the isolates are shown in Table 1. Bacillus spp. and E. coli have the highest occurrence of 90.24% and 85.36% respectively, while Salmonella spp. and Shigella spp. have the least occurrence of 26.82% and 30.48% respectively.

Total aerobic mesophilic bacteria count, the coliform and fecal coliform count of the morning and afternoon samples obtained from fast-food joints, street vendors and the laboratory-prepared samples are shown in Table 2,3 and

4.The high frequency of occurrence of Bacillus and E.coli in this study is in line with the report of Ankita[4]. He noted that this high incidence indicates poor hygienic conditions in the preparation of the salad vegetables. The isolation of Bacillus sp in the salad is not surprising as the spores of Bacillus are easily carried in air and dust, and if food is not properly covered, these spores could settle on the food, and once nutrients are present, the spores can actively grow and thrive on the food[13].

The total aerobic mesophilic bacteria count of morning samples were significantly lower than that of afternoon samples, in both street- vended samples and fast-food samples (Table 2). This could be as a result of lack of preservation facility. The street vendors that have refrigerators preferably use it for cooling drinks rather than salad. They simply store the fresh cut vegetable salad in a big bucket which will be easy for them to reach, open and sell to their demanding customers. Microorganisms can easily multiply and grow in such conditions. Also the fast-food joints that have enough refrigerators do not have steady power supply. This result in holding temperature fluctuation, and this permits the activities of microorganisms already present in the salad. Ameko et al.,[13] reported in their study on microbial safety of raw mixed salad, that enteric pathogens were found on their samples in the morning and afternoon, but generally, that contamination was significantly higher ($p<0.05$) in the afternoon than in the morning. The results obtained from fast-food joints samples gave lower aerobic mesophilic bacteria count, total coliform count and total fecal coliform count than street- vended samples (Tables 2, 3 and 4), while the laboratory- prepared sample gave the least count.

It was discovered that 44% of vendors do not take conscious precaution to avoid contamination of the raw salads during preparation and sale, and this is mainly due to the ignorance of majority of them on the causes and prevention of food contamination[13]. The fast-food joints have better structure, working environment, storage/ preservation facilities like refrigerator unlike the street vendors. The presence of E. coli and increasing fecal coliform count, ranging from 2.60 Log_{10} CFU/g to 4.90Log_{10}CFU/g is indicative of fecal contamination. It can enter through poor hygiene and unhealthy nature of food handlers during preparation or processing, and the use of water already contaminated with E. coli for washing the vegetables. The laboratory prepared salad samples were more wholesome, bruise-free and healthy.

Hazard Analysis Critical Control Point – Technical Quality Management (HACCP-TQM) guidelines gave threshold and quality levels for food borne illness hazards. The suggested level of E. coli at purchasing of vegetables for salad is 1.0Log_{10}CFU/g[8]. In this study, 12(14.63%) samples fall below this purchase limit while 70(85.37%) of the samples exceeded this limit. Faecal coliform limit for vegetable eaten uncooked has been stated to be ≤ 3.0Log_{10}CFU/g by International commission of Microbiological Specification for food[14]. In this study, the total coliform bacterial counts in 80 (95%) were found to be > 3Log_{10}CFU/g. Due to the increase in the number of outbreaks of food borne diseases in connection with

consumption of fresh produce, the world health organization has recommended that edible crops should be irrigated only with biologically treated effluent that has been disinfected to achieve a coliform level of not more than 100 cells/100ml in 80% of the samples. This is because, the use of untreated waste water to irrigate garden has been implicated as one of the important sources of contaminating salad vegetable. However, one major way of preventing the ingestion of pathogens present in salad vegetables is by washing the vegetables in 10 % brine (salt solution), this will tie up available water in the vegetables, making it present, but not available for the pathogenic microbes. Moreover, refrigeration of the salad vegetables prior to processing within a holding time is required to avoid long time exposure to aerial microbes.

Table 1. Prevalence of Bacterial Isolates in Vegetable Salad Samples

solates	Frequency of isolation	Percentage
occurrence (%)		
Salmonella spp.	22	26.82
Shigella spp.	25	30.48
E. coli	70	85.36
Staphylococcus spp.	58	70.73
Bacillus spp.	74	90.24
Proteus spp.	54	54.87
Serratia spp.	39	47.56
Streptococcus spp.	64	78.4
Micrococcus spp.	52	63.41
Pseudomonas spp.	33	40.24
Klebsiella spp.	57	69.51
Total number of samples examined = 82		

Table 2. Total aerobic mesophilic bacterial count of mixed vegetable Salad Samples Log_{10} (X±SD) i.e. Logarithm Value of the Mean± Standard Deviation /g

Location	Xm	Xa	Ym	Ya	Zm	Za
1	4.63±0.31	4.87±0.26	5.50±0.46	6.75±0.30	3.50±0.21	3.54±0.46
2	4.68±0.33	4.78±0.43	5.57±0.55	6.60±0.27		
3	4.58±0.07	4.69±0.33	5.61±0.57	6.51±0.21		
4	4.74±0.57	4.79±0.57	5.67±0.49	6.61±0.52		
5	4.69±0.27	4.86±0.33	5.56±0.31	6.94±0.38		
6	4.15±0.51	4.70±0.46	5.80±0.14	5.59±0.51		
7	4.38±0.38	4.93±0.21	6.72±0.33	7.66±0.57		
8	4.21±0.21	4.69±0.52	5.78±0.46	6.75±0.06		
9	4.46±0.46	4.88±0.15	5.17±0.38	6.98±0.33		
10	4.40±0.40	4.98±0.38	5.16±0.33	6.74±0.45		

Key:

Xm = Salad samples obtained from fast-food vendors in the morning and analysed.

Xa = Salad samples obtained from fast-food vendors in the afternoon and analysed.

Ym = Salad samples obtained from street vendors in the morning and analysed.

Ya = Salad samples obtained from street vendors in the afternoon and analysed.

Zm = Salad samples prepared in the laboratory and analysed in the morning.

Za = Salad samples prepared in the laboratory and analysed in the afternoon.

Table 3. Total Coliform bacterial counts of the Vegetable Salad Samples Log_{10} (X±SD) i.e. Logarithm Value of the Mean± Standard Deviation/g

Location	Xm	Xa	Ym	Ya	Zm	Za
1	3.43±0.27	3.49±0.39	3.63±0.45	4.43±0.38	2.60±0.30	2.77±0.46
2	3.51±0.57	3.54±0.21	4.32±0.33	4.85±0.27		
3	3.20±0.06	3.34±0.46	3.77±0.06	4.63±0.26		
4	3.27±0.49	3.47±0.14	3.74±0.51	3.86±0.33		
5	3.44±0.38	3.57±0.57	5.47±0.51	6.73±0.57		
6	3.49±0.14	3.56±0.38	3.96±0.21	4.43±0.38		
7	3.47±0.06	3.51±0.21	4.46±0.27	4.86±0.14		
8	3.50±0.21	3.63±0.66	3.65±0.30	3.64±0.45		
9	3.39±0.38	3.55±0.38	3.51±0.46	3.94±0.46		
10	3.43±0.57	3.46±0.31	3.71±0.55	4.49±0.21		

Key:

Xm = Salad samples obtained from fast-food vendors in the morning and analysed.

Xa = Salad samples obtained from fast-food vendors in the afternoon and analysed.

Ym = Salad samples obtained from street vendors in the morning and analysed.

Ya = Salad samples obtained from street vendors in the afternoon and analysed.

Zm = Salad samples prepared in the laboratory and analysed in the morning.

Za = Salad samples prepared in the laboratory and analysed in the afternoon.

Table 4. Total fecal coliform bacteria counts of the Vegetable Salad Samples Log_{10}(X ±SD) ie. Logarithm Value of the Mean ± Standard Deviation

Location	Xm	Xa	Ym	Ya	Zm	Za
1	2.27±0.21	2.90±0.51	2.77±0.39	3.60±0.45	-	-
2	2.69±0.39	3.00±0.55	3.90±0.21	3.47±0.31		
3	-	-	2.77±0.30	3.84±0.38		
4	2.60±0.27	2.84±0.14	2.95±0.49	3.07±0.14		
5	-	-	4.00±0.57	4.90±0.57		
6	2.90±0.26	2.95±0.45	2.69±0.06	3.84±0.38		
7	2.69±0.57	2.60±0.33	3.90±0.49	3.60±0.27		
8	-	1.30±0.06	2.84±0.26	2.95±0.33		
9	2.60±0.26	2.47±0.21	2.30±0.38	2.69±0.06		
10	2.84±0.38	3.04±0.46	3.04±0.57	3.84±0.49		

Key:

Xm = Salad samples obtained from fast-food joints in the morning and analysed.

Xa = Salad samples obtained from fast-food joints in the afternoon and analysed.

Ym = Salad samples obtained from street vendors in the morning and analysed.

Ya = Salad samples obtained from street vendors in the afternoon and analysed.

Zm = Salad samples prepared in the laboratory and analysed in the morning.

Za = Salad samples prepared in the laboratory and analysed in the afternoon.

- = No colonial count obtained.

DISCUSSION

This study revealed the presence of potentially pathogenic microorganisms in the vegetable salads examined. The results highlighted the importance of proper and adequate precaution during processing and sale of vegetable salad. Minimal holding time before consumption of prepared salad is recommended.

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