*Available: DOI: https://doi.org/10.54117/gjpas.v1i1.23* *Research article*

**Gadau Journal of Pure and Allied Sciences**
*Gadau J Pure Alli Sci, 1(1): 68-72 (2022)*
*ISSN: 2955-1722*
*DOI: https://doi.org/10.54117/gjpas.v1i1.23*

# Bacteriological assessment on ready-to-eat salad sold in some fast food centers in Azare town, Bauchi State, Nigeria

Aishatu Abubakar* and Tabitha Joshua

Department of Biological Sciences, Faculty of Science, Bauchi State University, Gadau. P.M.B 06, Gadau, Nigeria.

*Correspondence: aishatuabubakar@basug.edu.ng

**Abstract**

Eating food that is contaminated by bacteria is one of the main reasons for food poisoning. Food poisoning can be caused by several bacterial species that are present on the raw vegetables and fruits and also in the dairy products that are used in the salad dressings and toppings. This study was carried out to isolate and identify pathogenic micro-organisms associated with ready-to-eat salads obtained from selected fast food centers in Azare town of Bauchi State, Nigeria. Samples of salads were collected from randomly selected locations within the town and subjected to microbial culture in Nutrient and MacConkey agar media for isolation of bacteria. Three bacterial species were isolated, namely; *Salmonella* Spp, *Escherichia coli* and *Staphylococcus aureus*. The total colony count ranged from $3.01x10^1$ cfu/g to$1.5x\ 10^3$ cfu/g respectively. The order of increasing colony count of bacteria isolated was *Salmonella* Spp, *E. coli*, and *Staphylococcus aureus*. The results from the studies showed that ready-to-eat salads samples obtained from the fast food centers have high microbial load and as such do not meet bacteriological standards. Therefore, consumption of such products may pose public health problem. It is therefore recommended that the total hygiene and sanitary conditions under which those fast foods centers operates should be monitored by the relevant Government agencies and stringent supervision of processing methods are applied.

**Article History**

Received: 24/05/2022
Accepted: 26/07/2022
Published: 30/07/2022

***Keywords***
Ready-To-Eat; Salad; Bacteriological Assessment; Pathogenic micro-organisms; *Escherichia coli*; *Staphylococcus aureus;* Food



**How to cite this paper:** Abubakar A. and Joshua T. (2022). Bacteriological assessment on ready-to-eat salad sold in some fast food centers in Azare town, Bauchi State, Nigeria. *Gadau J Pure Alli Sci, 1(1): 68-72*. https://doi.org/*10.54117/gjpas.v1i1.23*.

## 1.0 Introduction

Salad is a form of food made primarily of a mixture of raw vegetables and or fruits (Rajvanshi, 2010). Common vegetables used in salad include cucumber, pepper, tomatoes, onions, cabbages, and carrots. Other ingredients such as hard-boiled eggs, cheese, and meat are sometimes added to salads. However, the salads containing raw vegetables may be unsafe, mainly because of the environment under which they are prepared and consumed (Taban and Halkman, 2011).

Vegetables are well-known sources of useful nutrients in the form of vitamins, minerals, dietary fibers, and other phyto-nutrients including flavonoids, carotenoids, and phenolic compounds that may lower the risk of cancer, heart disease and other illnesses (James and Ngarmsak, 2011).Vegetables have been associated with out-breaks of foodborne disease in many countries (Halablab *et al.*, 2011). Organisms involved include bacteria, viruses and parasites (De-Roever, 1999). The outbreaks vary in size from a few persons affected to many thousands (Halablab *et al*., 2011). Contamination of vegetables may take place at all stages during pre and post-harvest techniques (Halablab *et al.*, 2011). Unsafe water used for rinsing vegetables and sprinkling to keep them fresh is also a source of contamination (Mensah *et al.*, 2002). Other possible sources of microorganisms include soil, feces (human and animal origin), animals (including insects and birds), handling of the product, harvesting and processing equipment and transport (Johannessen *et. al.*, 2002).

Vegetables that are eaten raw without any heating during the processing stages can get contaminated because of the manure and the irrigation water used in cultivation

(Pesewu *et al.*, 2014). Also bad and un-hygienic raw-mixed vegetable salad preparation methods can introduce various bacteria and parasites into the salad and cause diarrhea diseases. Literature has shown that eating food that is contaminated by bacteria is one of the main reasons for food poisoning. Food poisoning can be caused by several bacterial species that are especially present on the raw vegetables and fruits and also in the dairy products that are used in the salad dressings and toppings. (Beuchat *et al.*, 2006).

Microbes associated with fresh-cut fruit and vegetable products can vary greatly in accordance with the produce type and storage conditions. Temperature plays a significant role in determining the nature of the micro-flora associated with refrigerated fresh-cut fruit and vegetables (James and Ngarmsak, 2011). Several food borne bacteria such as *Campylobacter jejuni*, *Escherichia coli*, *Clostridium perfringens*, and species of *Salmonella, Staphylococcus*, *Yesinia* and *Shigella* have been previously reported to be the main pathogens which can contaminate vegetables salads and cause diarrhea after consumption of these contaminated vegetables salads (Beuchat *et al.*, 2006).

Microbial contaminants such as bacteria constitute the major cause of severity ranging from mild indisposition to chronic or life threatening illness or both (Ibrahim *et al.*, 2013). The implications of the microbial contamination and growth on vegetable produce include spoilage, decreased sensory appeal and decreased shelf life. However, coliforms are expected to be present in many raw foods and food ingredients of animal and plant origin (Bibek, 2005). Also, the occurrence of some coliforms of non-fecal origin and their ability to grow in many foods reduces the specificity of coliforms as an indicator of fecal contamination in raw foods (Bibek, 2005). However, coliforms are commonly used as bacterial indicator of sanitary quality of foods and water and are also utilized as indicators of microbial pollution as they are common inhabitants of both animal and human guts (Tortora, 1995).

Safe food is basic human right despite this, many foods are frequently contaminated with naturally occurring pathogenic micro-organisms, such pathogens cannot be detected organoleptically, but can cause diseases of varying severity including death especially in the way they are conserved during exposition for sales which provides condition for those micro-organisms to grow and reach considerable levels of contamination. Thus, food safety issues are of major important issues to the World Health Organization.

This research will bring to light the type and source of bacterial contamination in the salad sold in Azare town. This will help in taking measures to avoid the contamination and promote the bacteriological quality of the salad. The study will also be a reference point for further research on bacteriological quality of salad in Bauchi and other states in Nigeria.

## 2.0 Materials and methods

### 2.1 Description of the study area

The research study was carried out at Azare which is located at 11°40′27″N 10°11′28″E Coordinates: 11°40′27″N 10°11′28″E, at an elevation of 436 meters. The population has grown from 69,035 at the 1991 census to its 2007 estimated value of 110,452. In the last five years, the population has grown by more than 20%. It is also the largest growing town in the state and region. Growth is prominent in all directions with the creation of Bamako to the South-East, Unguwar Dankawu and Makara-huta Inuwa Dahiru Road to the North, Federal Lowcost-GRA to the Northwest and the extended growth in the south that sees the town engulfing places like Chilankori and Chara-Chara Yelwa.

The area is a place that has a fertile land for good agricultural activities the main crops found in the area are millet, groundnut, maize, beans, wheat, cottons, vegetables, Dogonyaro (Bishiyar Maina) and rearing of animal. It's the largest of several nearby towns in the region including Jama'are, Misau, Bulkachuwa, Disina, Faggo, Zadawa, and Madara.


**Figure 1: Study location of Azare town Katagum. (Source: Google maps)**
**Key:** ----●--- Azare

### 2.2 Sample collection

Ready to eat salad samples were collected from three fast food centers designated 1, 2, and 3 at randomly selected location in Azare town. Each of the samples was collected in morning, placed in sterile beaker. The samples were then taken immediately to microbiology laboratory for analysis; caution was taken not to keep the samples in the refrigerator for too long.

### 2.3 Isolation of bacteria

From each sample 1 gram was weighed out and it was homogenized in 9m/s of peptone water. This gives a 1:10 or $10^{-1}$ dilution, done in aseptic condition. It was shaken and allowed to stand on the bench for 5 minutes. A dilution to about $10^{-6}$ was made of sterile peptone water, making sure suspension for inoculation were obtained from each dilution by pipetting from the clear middle layer after the pieces of the vegetables has settled down (Adesiyun *et al.*, 2005).

**2.4 Total aerobic plate count**

From each dilution 1.0m/s was dispensed into sterile Petri-dish and 25mls of nutrient agar was added, which was allowed to gel. The plates were incubated at $37^0$C for 24 hours. All colonies that grew on the agar are considered, then counted and expressed as colony forming units per gram (cfu/g) (Adesiyun *et al.*, 2005).

**2.5 Total coliform count**

From each dilution 1.0m/s was dispensed into sterile Petri-dish and 25mls of MacConkey agar was added, which was allowed to gel. The plates were incubated at $37^0$C for 24 hours. All coliforms that appear pinkish ranging from small to medium in the agar are counted and expressed as colony forming unit per gram (cfu/g) (Adesiyun *et al.*, 2005).

**2.6 Isolates characterization**

Isolates were characterized on the basis of their cultural features (i.e. colour, shape, edge, elevation and so on). Morphological features (such as motility, gram-reaction, cell arrangement and shape) and biochemical features (Adesiyun *et al.*, 2005).

**2.7 Morphological characteristics**

**2.7.1Gram staining and microscopy**

From each plate that indicate positive growth, a discrete colony was isolated and smeared on a glass slide with a drop of normal saline put on this slide for viewing. The smear was air dried and gram stained and crystal violet stain was applied for one minute, and then washed off with clean water. Lugol's iodine was rapidly added for one minute and washed off. The smear was decolorized using acetone and washed immediately and then safranine was added for two minutes and washed off with clean water. The slides were air dried and observed under the microscope using oil immersion objective (x100) and the gram reaction was observed and recorded (Adesiyun *et al.*, 2005).

**2.8 Biochemical test**

**2.8.1 Motility test**

The test organism was inoculated into peptone water and then incubated aerobically at $35^o$C overnight. After overnight incubation, a drop of the bacteria culture broth was placed in the center of a clean, grease free slide and covered with a clean Vaseline bound cover slip. The prepared slide was observed under microscope using x40 objective with the condenser iris closed sufficiently to give good contrast (Adesiyun *et al.*, 2005).

**2.8.2 Oxidase test**

This test helps in identifying the enzyme called oxidase produced by microorganisms. Procedure: a piece of filter paper was soaked in a few drop of oxidase reagent (tetramethyl-p-phenylenediamine dichloride). A colony of the test organism was then smeared on a soaked filter paper. An oxidase producing organisms on the filter paper oxidized the phenylenediamine in the reagent to deep purple colour (Adesiyun *et al.*, 2005).

**2.8.3 Catalase test**

This test is used to demonstrate the presence of enzyme catalase, which catalysis the release of the oxygen from hydrogen peroxide. Procedure: The pure culture of the test organism was placed and added to a drop of 3% hydrogen peroxide solution on a clean slide. The production of a gas bubble from the surface indicates positive result (Adesiyun *et al.*, 2005).

**3.0 Results and Discussion**

The most predominant bacterial pathogens isolated in the present study include *Staphylococcus aureus*, *Escherichia coli* and *Salmonella.* The isolation of similar pathogens has also been reported by previous workers from various foods (raw and ready-to-eat foods) (Fang *et al.*, 1999). According to the international commission for microbiology and specification for foods states that $0\text{-}10^3$ is acceptable, between $10^4$ less than or greater than $10^5$ is tolerable, and $10^6$ and above is unacceptable.

The difference in the level of contamination could be due to difference in personal and general hygiene in relation to the handling practice of the producers and the environment. The overall mean of Total aerobic plate count (TAPC) 6.60 cfu/g deduced for salad obviously shows that the level of contamination was higher than the acceptable levels which are considered good quality and reverse is the case if higher than the acceptable levels which is attributed to unhygienic and unsanitary conditions employed in the course of preparation and after preparation Odu and Ameweiye (2013). The total plate count and total coli form count obtained from the samples could also be attributed to environmental contamination as it plays enormous role in influencing the bacterial load since salads are prepared in open placed, using bare hands and sometimes expose to air during sales.

The characteristics and colonial morphology of each organism isolated were in which some of the organisms are gram positive and some gram negative, some of the organisms grew on nutrient agar having different colour and shape and also some on Mac-Conkey agar with different shapes and colour. The biochemical tests include catalase, citrate, oxidase and so on. Some of the isolates were found to be gram negative and some gram positive. Also some are gram positive and some gram negative in some of the biochemical test, whereas all are found to be oxidase negative.

**Table 1: Prevalence species in ready-to-eat salad from three fast food centers in Azare town**

| Fast food centers | Bacteria isolates and their percentages (%) | | |
|---|---|---|---|
| | ***E. coli*** | ***Salmonella typhea*** | ***Staphylococcus aureus*** |
| 1 | 60**.0** | 40**.0** | No growth |
| 2 | **35.5** | 26**.0** | No growth |
| 3 | **No growth** | No **growth** | 25**.0** |

**Table 2: The mean values of bacteria counts (cfu/gx10$^3$) in three different fast food centers using different media**

| Fast food center | Bacteria Counts | |
|---|---|---|
| | **MacConkey Agar** | **Nutrient Agar** |
| 1 | $3.1\times \mathbf{10^1}$ | $2.0\times \mathbf{10^1}$ |
| 2 | $2.4\times \mathbf{10^2}$ | $1.5\times \mathbf{10^2}$ |
| 3 | $1.7\times \mathbf{10^3}$ | $1.0\times \mathbf{10^3}$ |

**Table 3: Cultural characteristics, colonial morphology and inference of the identified pathogens**

| FFC | Nutrient Agar | Mac-conkey Agar | Gram Reaction | inference | |
|---|---|---|---|---|---|
| 1 | Round, smooth Creamy, white-colonies | | + | *Staphylococcus aureus* | Yellow |
| 2 | | Circular, moist, smooth flat and pink Colonies. | - | *Escherichia c* | |
| 3 | | Colorless and Transparent | - | *Salmonella typhae* | |

**Key:** - =Negative; + =Positive, FFC; fast food centers

**Table 4: Biochemical characteristics of isolate obtained from ready-to-eat salad**

| Fast food Centers | Microscopy | Gram stain | Catalase | Oxidase | Motility | Isolate |
|---|---|---|---|---|---|---|
| **1** | **cocci** | **+** | **+** | **-** | **-** | ***E. coli*** |
| **2** | **Rods** | **+** | **+** | **-** | **+** | ***S. typhae*** |
| **3** | **Rods** | **-** | **+** | **-** | **-** | ***S. aureus*** |

**Table 5: Bacteria isolated from the ready-to-eat salads in the three fast food centers**

| Bacteria spp | Fast food centers | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| *Eschirichia coli* | _ | _ | NG |
| *Staphylococcus aureus* | NG | NG | + |
| *Salmonellatyphae* | NG | + | NG |

**Key: -** = Negative; + =Positive; NG = No Growth

In which some of the organisms are found in all the samples where as one organism was found in 2 out of the 3 samples.

Contaminated food and water are the major sources by which the bacterium is spread. Selected strains can cause a wide variety of infections in hospitals and community settings (Donnenberg, 2005). These include diarrheal illness, urinary tract infections, meningitis, sepsis, wound infections, nosocomial pneumonia and dysentery. A subgroup called Enterohaemorrhagic *Escherichia coli* (EHEC) can cause food borne illness as the *Escherichia coli* 0157:H7 strain which cause severe and potentially fatal illness known as *Haemorrhagic colitis* which is characterized by bloody diarrhea and severe abdominal pain (Dolores and Doyle, 2001). *Escherichia coli* is commonly used as a surrogate indicator; its presence in food generally indicates direct or indirect fecal contamination. However, in Nigeria, a number of foods have been reported to have a high incidence of the bacteria (Adeyisun, 1995; Okonko *et. al.*, 2009).

**4.0 Conclusion**

This study has demonstrated that the ready-to-eat salads sold in the study site do not meet the required quality and safety levels. Some of the bacteria isolated especially *Staphylococcus aureus, Escherichia coli* and *Salmonella specie* that were identified in each and every collection of the food sample are potential enteric pathogens and are known to cause gastroenteritis. This clearly depicts poor handling and management leading to cross contamination as *S. aureus* is a normal flora of the skin and *E. coli* demonstrate fecal contamination.

**References**


Adebayo-Tayo, B.C., Onlude, A.A., Ogunjobi, A.A. (2006). Bacteriological and proximate analysis of periwinkle from two different creeks in Nigeria. *World Applied Science Journal* 1(2): 87-91.

Adesiyun, A.A, Umoh, J.U, Mosimabale, F.O kwaga, J.K.P, Dusai, D.H.M, Okolocha, E.C, Ajogi, A.I, Dzikwi, A.A, Egege, S.C, Oni, O.O, Lombin, L.H, Ezeifeka, G.O, Luga, I, Kabir, J. (2005). Manual for clinic in veterinary public Health and Preventive Medicine. Ahmadu Bello University, Zaria. *ABU* press limited. p11-14.

Adesiyun, A.A. (1995). Bacteriological quality of some Trinidadian ready-to-eat foods and rinks and possible health risk to consumers. *Journal for Food Protection.* 58(3): 651- 655.

Beuchat, L. R. (2006). Vectors and Conditions of Pre-harvest Contamination of Fruits and Vegetables with Pathogens Capable of Causing Enteric Diseases. *British Food Journal.* 108 (1): 38-53.

Bibek, R. (2005). Fundamental Food Microbiology, *CRC Press. London.* p 625.

De Roever, C. (1999). Microbiological safety evaluations and recommendation on fresh produce. *Journal for Food Control.* 9: 321-347.

Dolores, G.E., Doyle, J.G. (2001). *Escherichia coli* in diarrhea disease. Annual.*International Medicine.* 247: 81-90.

Donnenberg, M.S., Mandel, G.L., Bemet, J.E., John, R., Mandel, D. (2005). *Enterobacteriaceae* Principles and Practices of Infectious Diseases. 6th Ed., pp. 267-286.

Fang, T.K., Chen, C.Y., Kuo, W.Y. (1999). Microbiological Quality and Incidence of *Staphylococcus aureus* and *Bacillus cereus* in Vegetarian Food Products. *Food Microbiology.* 16: 385-391.

Halablab, M.A., Sheet I.H. and Holail, H.M. (2011). Microbial quality of raw vegetables grown in Bekaa valley, Lebanon. *American Journal of Food Technology.* 6: 129-139.

Ibrahim, H., Moshood, A.Y., Tengku, H.A. (2013). Isolation and identification of Bacteria associated with balangu (roasted meat product) sold in Bauchi, Nigeria. *IOSR Journal for Pharmaceutical Microbiology.* 2(6): 38-48.

James, J.B. and Ngarmsak, T. (2011). Processing of fresh cut tropical fruits and vegetables*:* A technical guide. *FAO, Bangkok, p* 102.

Johannessen, G.S., Loncarevic S. and Kruse (2002). Bacteriological analysis of fresh produce in Norway. *International Journal of Food* H. *Microbiology.* 77: 199-204.

Mensah, P.D., Yeboah-Manu, K., Owusu-Darko S. and Ablordey, A. (2002). Street foods in Accra, Ghana: How safe are they? *WHO Bulletin*. 80: 546-554.

Okonko, I.O., Donbraye, E., Babatunde, S.O. (2009). Microbiological quality of Seafood processors and water used in two different sea processing plant in Nigeria. *EJEAF che* 8(8): 621-629.

Pesewu, G. A., Gyimah, I. K., Agyei, J. N. Y. K., Adjei, D. N., Olu-Taiwo, M. A., Asmah, R. H., and Ayeh-Kumi, P. F. (2014). Bacteriological Assessment of the Quality of *Brassica oleracea*var. *Capitata* Grown in the Accra Metropolis, Ghana. *African Journal of Microbiology. Res.* 8 (22): 2223-2228.

Rajvanshi, A. (2010). Bacterial Load on Street Vended Salads in Jaipur City, India. *Internet Journal for Food Safety.* 12: 136-139.

Taban, B. M., and Halkman, A. K. (2011). Do Leafy Green Vegetable and Their Ready-to-eat (RTE) Salads Carry a Risk of Food borne Pathogens? *Anaerobe.* 17(6): 286-287.

Tortora, G. (1995). Microbiology, The Benjamin Publishing Co. Inc. New York. p 532.