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**Microbial Quality of Ready-to-Eat Salad Sold in
Benin City, Southern Nigeria****Wogu, M. D. & Iwezeua, I.**

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E-mail: mddikewogu@yahoo.com**Abstract**

This paper examines the microbial quality of ready-to-eat vegetable salads obtained from three fast food centres in Benin City, Edo state, Nigeria. Across the counter samples of salad were collected from randomly selected locations within the city and subjected to microbial culture in Nutrient and MacConkey agar media for isolation of bacteria. Three bacteria species were isolated, namely; Salmonella spp. Escherichia coli and Staphylococcus aureus. The total colony count ranged from 3.01×10^1 cfu/g to 1.5×10^3 cfu/g. The order of increasing colony count of the bacteria isolates was Salmonella > E. Coli > Staphylococcus aureus. Results from the study shows that ready-to-eat-salad samples obtained from fast food centres in Benin City have high microbial load and as such do not meet bacteriological quality standards. Consumption of such products may pose public health problem. It is recommended that the total hygiene and sanitary

conditions under which these fast food centres operate should be monitored by the relevant government agencies and stringent supervision of processing methods are applied.

Key words: Salads, Microbial quality, food- borne infections, Benin City

Introduction

Salad is a dish of raw leafy green vegetables, often tossed with pieces of other raw or cooked vegetables, fruit, and cheese of other ingredients and served with a dressing. Dressing in this context is the sauces for salads, such as vinaigrette, Mayonnaise-based, egg yolk and gently heating (Grant, 2008). Salad is prepared by sliced or diced carrots, onions, cabbage, cauliflower, lettuce, beetroot and served as appetizer as the first course of meal. Also, it contains a portion of protein (Tjoa *et al.*, 2001). Ready-to-eat salads are foods that are processed and are ready to be eaten. They are sold at various food centres i.e. restaurants and hotels.

In Southern Europe, salad is generally dressed for the dinner with oil and vinegar. In Denmark dressing are often based in crème fraise which is part of cooked dressing. In France traditional dressings are vinaigrettes. In Eastern Europe countries and Russia mayonnaise is predominant. Finally in Asia, it is common to add sesame oil, fish sauce, and soy sauce citrus juice to salad dressing. The following are examples of common salad dressing; In the U.S. Market, fast food chains such as McDonald's KFC that usually sell "Junk Food" such as fries, fried chicken and hamburgers, now sell packaged salad to appeal to the health-conscious (Gillespie *et al.*, 2000).

Blue cheese dressing, Caesar dressing, extra virgin olive oil, French dressing, Honey Dijon, Italian dressing, Louise dressing, vinaigrette, etc (Goepfert and Klim, 1972). One of the advantages of salads is that the ingredients from which they can be made are large in number. The composition, as well as the total food value of salad depends entirely on the ingredients of which they are composed. An understanding of the composition of the ingredients used in salad enables one to judge

fairly accurately whether the salad is low, medium or high in food value and whether it is high in protein, fat, or carbohydrates. Some of the composition of salad are; pH between 2.5 and 4.5, phosphoric acid, sulfuric acid, hydrochloric acid, glutamic acid, maleic acid, betaine, tartaric acid, aspartic acid, oil phase comprises from 10 to 35% by weight of total compositions, Pectic, Xanthan gum, Carragenan etc.

Salad constitutes provide many health benefits. The following constituents are obtainable from a salad dressing with lettuce; protein 0.5g, fiber 0.7g, calcium 10mg, potassium 78mg, vitamin C 1.5mg, folate 16mcg, vitamin K 13.3mcg, beta carotene 104 mcg, lutein and zeaxanthin 152 mcg etc. Also parts of the salad contain a milky, white latex (sap) which when collected and dried, forms the drop known as lectucarium (G33) mostly found in lettuce acids citric, malic and oxalic (phenolic). Others include Coumarins, Aesculin, cichorin, flavonoids, mannitol, resins and sugars etc (International Standard Organization, 1973).

Salads with dark green vegetables are richer in nutrients. The amount of calories in lettuce for one serving is 8. Lettuce is low in saturated fat and there is practically no cholesterol in it. It is also a good source of dietary fiber. Nutritional benefits are enhanced due to folate present in it. We know that folate helps in fighting heart diseases, omega fatty acids in lettuce, which help in maintaining brain health.

Salad has high water content because of its dressing but it is low in calories. Hence, it is used by people who are aiming at weight loss, help in disorders and strokes. Iceberg lettuce in salad is low in sodium. It contains no saturated fats and cholesterol; hence it is seasoned with salt or with some type of dressing. It also contains 1% of calcium, 7% of vitamin A, 3% of vitamin C, and 2% of iron of the recommended daily allowance "RDA" (Pragya, 2010).

Microorganisms are present in natural ecosystems such as air, soil and water. They are also present in and on other living organisms including plant (source). Therefore, all processed foods may or may

not be contaminated by microorganism. However, food poisoning outbreaks caused by fresh salad vegetables have been making food safety headlines on a regular basis in recent years, especially in the USA. A series of large, high profile *Salmonella* sp. and *E. coli* outbreaks linked to spinach and lettuce has damaged the US fresh produce industry and left the sector with a reputation for high-risk products. Research is beginning to reveal complex relationships between microbes and plants, which seem to play an important role in the contamination process. In the future, it may be possible to use this knowledge to devise innovative new way to minimize or even prevent contamination with food borne pathogens.

A recent comprehensive review by the UK Health Protection Agency of prepared salad provides some of the answers. The review authors looked at more than 200 general outbreaks of food borne diseases that were recorded in England from 1992 to 2006 and found that only 82 (4%) of them were associated with prepared salads. The review also pointed out that most of the outbreaks linked to salads occurred in the catering sector and were associated with infected food handlers, cross contamination and poor storage. Just two outbreaks were associated with salad sold by retailers. However, they also found that these outbreaks, especially those associated with lettuce tended to be larger than those caused by other food types and more prolonged.

There has been some analysis of food borne disease outbreaks in the USA between 1973 and 2005 and these showed that “fresh plant produce” was responsible for only 0.7% of outbreak in the 1970’s but that this had risen to 6% by the 1990s. While some of this increase can be accounted for by rising consumption, the figures show that between 1986 and 1995, the number of food borne disease outbreak linked specially to leafy green rose by 60%, but consumption rose by only 17%. The trend from 1995 to 2005 was similar, pointing to a growing problem with the safety of vegetables like lettuce and spinach. Cross contamination include: Using the same knife or chopping board to cut raw meat and ready-to-eat foods (salad, cooked

quiche etc), defrosting food or placing dirty utensils and equipment in the hand wash basin, storing raw food above ready-to-eat food.

Traditionally, the main decontamination method available to the produce industry has been washing with potable water, often chlorinated. Washing with chlorinated water is routinely applied to leafy green vegetable (salad) like lettuce especially for pre-packed ready-to-eat salads but it's effectiveness is limited (Dawson and Carnet, 1991; Grant *et al.*, 2008). Some genera of bacteria found in salad include *Aeromonas* sp., *Bacillus* sp., *Campylobacter* sp., *Clostridium* sp., *Citrobacter* sp., *Escherichia coli*, *Listeria*, *Leuconostoc* sp., *Pseudomonas* sp., *Shigella* sp., *Salmonella* sp., *Staphylococcus* spp, *Proteus* sp, *Zanthomonas* spp. etc.

There is paucity of information on food borne disease and their transmission in ready-to-eat food centres located in different parts of Benin City. Many people patronize these assorted vegetable salads presently. Hence there is a strong need for a scientific investigation of the microbial quality of ready-to-eat salad from food centres. This study should present information on possible microbial contaminants which may be of public health importance.

Aims and Objectives

The aims and objective of this study are to:

1. Identify the microorganisms present in pre-package mixed vegetable salads from different retail outlets in Benin City, Edo State.
2. Investigate the antibiotics sensitivity pattern of the microorganisms isolated from vegetable salad.
3. Determine the prevalence of pathogenic microorganisms present in ready-to-eat salad.

Materials and Methods

Samples Collection

Ready-to-eat salad samples were collected from three fast food centres (designated 1, 2, 3) located at randomly selected locations in Benin City. Each of the samples were collected in the morning, placed in sterile plastic packs and taken to the laboratory for analysis.

Sample Analysis

From each salad sample, 10g weighed and ground to paste using mortar and pestle and later transferred into different conical flasks each containing nutrient broth and agitated in a shaker for twenty minutes. The homogenized samples were left to stand for two hours in order to resuscitate the microorganisms present.

The samples were subjected to pour plate method in nutrient agar and MacConkey agar media for total viable bacteria and coliform counts.

Standard procedure for isolation and characterization of bacteria colonies was used in the study. Bacteria colonies were purified by replicating on nutrient agar using streaking method with an inoculation loop and incubated for 24 hours at 37°C. Having obtained pure cultures, further characterization with biochemical tests namely; catalase, coagulase, oxidase, indole, citrate utilization and sugar fermentation was done.

Results

Samples of ready-to-eat salad in three different fast food centres namely; Mr. Biggs. Mat-ice and kada fried Chicken in Benin City, Edo State were examined for microbial quality.

The bacterial species isolated from the salad samples belong to 3 genera. Two genera representing 60% of the isolates were gram negative while one gram positive genus accounted for 40% (Table 1). A total of 2 of the salad samples studies were contaminated with *E. coil* and *Samonella* spp. While 1 was contaminated with *Staphylococcus aureus*. Salad samples obtained from Fast Food

Center 1 had the highest contamination rate of 78%, samples from Fast Food Center 2 had 52%, while samples from Fas Food Center 3 recorded 40%.

The bacteria isolates obtained in three fast food centres are presented in tables 1 and 2.

The prevalence of human pathogens isolated from ready-to-eat salad represented in percentage is shown in Table 1.

Table 1: Prevalence species in ready-to-eat samples from three restaurants in Benin City

	BACTERIA ISOLATED AND THEIR PERCENTAGES (%)		
Fast Food Centers	<i>E. coil</i>	<i>Salmomella</i> spp	<i>Staphylococcus aureus</i>
1.	60	40	NG
2.	35.3	26	NG
3	NG	NG	25

Key: NG= No growth

Plate count and colonies observed were counted and recorded after incubation at 37°C for 24hours to get the total colony count in cfu/g (Table 2).

Table 2: The mean values of bacteria counts (cfu/gx10³) in three different fast food centres using different media

	BACTERIA COUNTS ON	
Fast Food Centers	MacConkey Agar	Nutrient Agar
1.	3.1 x 10 ¹	2.0 x 10 ¹
2.	2.4 x 10 ²	1.5 x 10 ²
3	1.7 x 10 ⁴	1.0 x 10 ³

The identification and characterization of bacteria isolates based on their cultural characteristics, morphological characteristics and biochemical test are shown in Table 3.

Table 3: Morphological and biochemical characterization of bacteria isolates

Characteristics	Salad Isolates of Mr. Biggs	Salad Isolates of Mat-Ice	Salad Isolates of Kada
Diameter	5mm	4mm	3.4mm
Colour	Yellow	Deep Yellow	Cream
Opacity	Opaque	Opaque	Opaque
Elevation	Raised	Flat	Slightly elevated
Shape	Circular	Circular	Circular
Suspected gram stain characteristics	Gram-ve	Gram-ve	Gram+ve cocci in pairs
Small	Fishy small	Milky smell	Fishy smell
Margin	Smooth	Serrated	Smooth
Biochemical reaction	Coagulase-ve Catalase-ve	Coagulase-ve Catalase-ve	Coagulase+ve Catalase+ve
Surface appearance	moist	Moist	Moist
Organism suspected	<i>E. coli</i>	<i>Salmonella</i> spp	<i>Staphylococcus aureus</i>

Key: -ve=Negative; +ve=Position

The bacteria isolated from vegetable salad and their prevalence in the various fast food centers are shown in Table 4.

Table 4: Bacteria isolated in three fast food centres in Benin City

BACTERIA	RESTAURANT		
	MR. BIGGS	MAT-ICE	KADA
<i>E. coli</i>	-ve	-ve	NG
<i>Staphylococcus aureus</i>	NG	NG	+ve
<i>Salmonella</i> spp.	NG	+ve	NG

Key: -ve=Negative; +ve=Position; NG=No Growth

The sensitivity patterns of bacteria isolates to different commercial available antibiotics are shown in Table 5.

Table 5: Resistance to various antibiotics

Micro-organism	DRUGS							DRO
	CIP	CHL	PEX	GEN	OF	SIP	T	
<i>E. coli</i>	30mm	15mm	R	R	25mm	R	R	10mm
<i>Salmonella</i> spp.	15mm	R	R	10mm	R	R	16mm	R
<i>Staphylococcus aureus</i>	26mm	R	R	R	R	30mm	R	20mm

Key:

CIP = Ciproxin, CHL= Chlorphenicol, PEX= Pefloxacin, GEN = Gentomycin, OF = Ofloxacin, SIP = Siprosan, T = Torivid, DRO = Dovin, R = Resistant.

Discussion

The number of documented outbreaks of human infection associated with the consumption of raw vegetable (salads) has increased in recent years. According to a report by the Centre for Disease Control and Prevention (CDC, 1996) the number of salad related outbreaks per year doubled between the period from 1973 to 1987 and from 1988 to 1992. During both periods, the aetiologic agents were more than 50% of the outbreaks.

In this study, the objective was focused on the microbial quality of ready-to-eat salad. Three bacteria species were isolated namely; *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. The isolation of *Salmonella* sp. a human pathogen, in this study is very disturbing particularly as these samples were obtained from a very big fast food centre. In a similar study by CDC, outbreaks with identified aetiology were predominantly of bacterial origin with *Listeria* spp.,

E. coli serovars, *Staphylococcus aureus*, *Klebsiella* spp. and *Bacillus cereus* predominating. More recently, *Klebsiella* spp has been linked with infection of tomatoes, *E. coli* infection with lettuce and enterotoxigenic *E. coli* with carrots. Enteropathogens are among the human pathogens that pose the greatest risks when present on fresh vegetable. This is because of their potential to grow in salad prior to consumption and low infective dose as well.

In this study, the mean viable bacterial count in salad sample of Food Centre 1 was more than the mean viable bacterial count in salad sample from Food Centres 2 and 3 respectively. The high incidence of bacterial contamination of ready-to-eat salad reported in this study may partly be attributed to the large number of people (food handlers and cooks in the centres), who may not observe basic sanitation requirements for processing products that require no pre-heating before consumption. Another reason may be the non-availability of water in good quantity and quality for washing of fresh vegetables/fruits, and mass production of salads in big fast food centres.

According to Oni *et al.* (2004), Staphylococcal food poisoning, caused by enterotoxin- producing strains of *Staphylococcus aureus*, was the most common food borne illness. Sudden onset of symptoms occurs 30 minutes to 8 hours after eating contaminated food. The incubation period may vary in relation to individual's susceptibility, amount of toxin in the food, and amount of food ingested. The incidence of Staphylococcal food poison is much higher than the figure that was reported due to the short duration of illness (1-2days) and almost always self limited. Hence, many do not seek medical advice. However, deaths have been reported. Oni *et al.* (2004) reported that *Staphylococcus aureus* infection was the leading cause of food poisoning whereas in this study, *E. coli* was the largest group of bacteria isolated in the sample collected in the three fast food centres.

An outbreak of *Staphylococcus aureus* food poisoning was reported in the United States of America in 1977 by Robert (1982) after eating a baked ham with high salt content. Jones *et al.*, (2000) also reported

Staphylococcal food poisoning outbreak in the United States of America following the ingestion of a shredded pork barbeque and coleslaw bought from a convenience-market delicatessen. Other bacteria that were isolated in that study were *Salmonella* spp. (40%), *Staphylococcus aureus* (25%).

Dierick *et al.* (2001) has also reported *Bacillus cereus*, a well known cause of food borne illness that is not commonly reported because of its usually mild symptoms. However, he described a fatal family outbreak due to liver failure after the consumption of pasta salad. Spencer (1996) reported yeast food poisoning among two high school students who drank box juice containing high amount of yeast in forest trail middle school in park forest/Chicago heights school district. The presence of yeast was not seen because the product was not packed in a polyethylene bags. *Salmonella* spp. isolated in this study may be traced to contamination of vegetables by animal droppings used as manure. This finding is similar to a report by CDC (1996) in which disease causing pathogens were found to contaminate vegetables.

Also in this study, the antimicrobial sensitivity test was carried out on the salad samples and the bacteria isolated were found to be of public health significance, and were tested against the following antibiotics namely; Ciproxin, Chlorophenicol, Pefloxacin, Gentomycin, Ofloxacin, Siprosan, Tarcvid, Dovid, for gram positive and gram negative organisms respectively. The result shows the resistance profile of the bacteria isolated from the salad samples. In a similar study, Udo *et al.*, (2009) reported the antimicrobial resistance profile of potential human pathogens isolated from fresh vegetable salad in Calabar, South-South Nigeria. The isolate showed variable resistance profiles ranging from 25.71% to 81.82% for *Staphylococcus aureus* and *Klebsiella aerogene* respectively. Multiple resistance to antibiotics was observed in all the isolate except *Salmonella* spp.

In this study, the isolation of *S. aureus* and *E. coli* from salad samples indicates not only the preponderance of *S. aureus* on the skin but also the low level of standard in personal hygiene. The sensitivity tests on

the isolates showed that the resistance rate varied from of 25.71% to 81.82%. In this study an overall multiple resistance rate of 35.29% was recorded. This finding probably links the source of these isolates to humans who are commonly colonized by multiple drug resistant bacteria.

Conclusion

Ready-to-eat salads from standard fast food centres were found to have high microbial load. The result from this study also shows that the ready-to-eat salad samples examined did not meet bacteriological quality standards. Hence, it is recommended that a more stringent supervision to ready-to-eat salads in fast food centres should be carried out by relevant authorities.

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