Bacteriological Analysis of Salad Vegetable in Eke Awka Market, Anambra State, Nigeria

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I. INTRODUCTION

Vegetable salad is a very common food accompaniment in Nigeria. The vegetables that usually make up this recipe include tomatoes, cucumber, carrots, cabbage and lettuce. They are sold in almost every market, and can be seen hawked around by traders. Fruits and vegetable have been identified as significant sources of pathogens and chemical contaminants (Uzeh *et al.,* 2009). As a result, environmental and food microbiologists have continued to identify and suggest control measures for hazards at all stages in the supply chain (Johngen, 2005). Khan *et al,* (1992) reported that bacterial contamination results from various unsanitary cultivation and marketing practices, In another study, Tambekar *et al.* (2006) reported that bacterial contamination of salad vegetable 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, yeast and mould dominate the micro flora on row fruits and vegetable, the occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infection has also been documented (Hassan *et al.,* 2006).

Coli forms are facultative anaerobic Gram negative rods belonging to the family *Enterobacteriacaea*. They are known contaminants of food and water, causing various intestinal and extra-intestinal infections such as urinary, central nervous system and respiratory tract infections (John, 2007).

The presence of *E. coli* in some green leafy vegetables few studies have examined the presence of entophytes or surface associated bacteria from the perspective of human consumption, by sampling minimally processed vegetables such as ready-to-eat salad produce. Similarly, few studies have focused on the entire entophyte community, rather than just potential pathogens, even though native entophyte bacterial populations could potentially serve as competitors to such organisms.

However, in Nigeria, local utilization of carrots, cabbage, onions, and cucumbers is limited to direct unprocessed eating either wholly or a growing awareness on the need to evaluate microorganism associated with spoilage of these vegetables.

The aim of this research work is to isolate the organisms associated with Salad vegetable spoilage in Eke Awka market, Awka, Anambra state, Nigeria.

II. LITERATURE REVIEW

Shelf life of fresh-cut fruits and vegetable

Fresh-cut fruits and vegetable are any combination thereof that has been physically altered from its original form, but remains in a fresh state (IFPA, 2001). Fresh cut fruits and vegetable offer consumers ready to eat produce that is one of several convenient, nutritious and fresh-like tasting, and are a rapidly growing category of value-added produce products that are minimally or lightly processed. Processing of fresh-cut products involves sorting, cleaning, washing, heating, peeling, coring and slicing, depending on products. The processing can be as simple as fresh-cut grape tomatoes for which raw tomato fruit is only sorted and washed with sanitized water or as complicated as cut cantaloupe, for which cantaloupe is sorted, cleaned with brush and spray water, heat treated with hot water or steam, peeled, deseeded, chunked, and rinsed with sanitized water before packaging in rigid containers. The fresh-cut fruits include melon chunks and slices, cored and sliced pineapple, apple wedges treated with ant browning preservatives; peeled citrus fruits and segments; de-capped strawberry; de-stemmed and washed grapes; sliced kiwifruit and fruit salads. Examples of fresh-cut vegetable are shredded lettuce; shredded and diced cabbage washed and trimmed spinach, peeled "baby" carrots, cauliflower and broccoli florets, sliced and diced tomatoes, peeled and sliced potatoes, snapped green beans, trimmed green onions, cleaned and diced onions, and mixed salads. Compared with whole fresh produce, fresh-cut produce is ready-to-us (ready to eat), contain 100% usable product, and always requires processing, refrigeration (including chilling-sensitive). Fruit and vegetables that can be injured after a period of exposure to chilling temperatures. Fresh-cut produce or products are in a raw state or fruit-like nutritious, and contain live tissues without freezing, canning (heat sterilization), dehydrating, fermentation, acidification , or treatments with additives or processed, minimally processed refrigerated, lightly processed, and prepared in literature and application communications. Fresh cut fruit and vegetable sales are approximately & 12 billion per year in the North American food services and retail market and account for nearly 15% of all produce sales (IFPA, 2001). Fresh-cut produce offer growers an opportunity to increase sales by adding value to raw agricultural commodities. The largest portion of US fresh-cut vegetable sales at retail is fresh-salads.

Impact of microbiological spoilage

Microbiological spoilage or microbiological shelf life has become a major reason for sensory quality shelf life failure for most package fresh-cut fruits and vegetables, followed by surface

discoloration (e.g., pinking of cut lettuce, browning of cut potato, graying or browning with processed pineapple and gray discoloration with cabbage), water soaked appearance or translucency (e.g., cut water melon, papaya, honey dew and tomatoes), moisture loss (e.g., "baby" carrot and celery sticks), off-aroma (e.g., diced cabbage), flavor changes(e.g., cut kiwi fruits). Microbial spoilage including off-flavor (e.g., fermented aroma with cut lettuce, sour taste with cantaloupe and bell paper) formation, slimy surface (e.g., "baby" carrots), wetness and soft rot and visual microbial growth/colonies has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products (Sapers *et al.,* 2001).Bracket (1994) concluded that microbial decay can be a major source of spoilage of fresh-cut produce. O'Connor Shaw *et al.,* (1996) reported that microbial spoilage is a limiting factor for shelf life of fruit pieces stored under controlled temperature or atmospheric conditions. Shelf life, including microbial spoilage, results in 30-50% shrinkage of fresh-cut fruit (Warren, 2005) microbial spoilage has used by quality failure for more than 50% of fresh–cut vegetable produce commodities and almost have been treated with preservative (such as anti browning reagent) or packaged properly using MAP technologies. Under equilibrium modified atmosphere (MA) conditions, mixed fresh-cut bell pepper (including green, yellow, and red bell pepper) was unacceptable by day 6 of storage at 7^{o}C due to acidic flavor, water loss and texture change (Jacxsens *et al.,* 2003). Processed lollo Rosso lettuce had a shelf life of shorter than 7 days at 5^{o}C due to high microbial counts and off-odor formation under MAP.

Sources of Microbial Contamination

Microbial contamination sources of fresh-cut fruits and vegetables include raw materials and contact with processing equipment. The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruit and vegetables Sapers' *et al.,* (2001) reported that, compared with the good surface sanitization practices, no decontamination treatment or an ineffective antimicrobial treatment on whole cantaloupe resulted in premature microbiological spoilage of fresh-cut cantaloupe studies have also revealed over al-year period of sampling that there is a close relationship between the total mesospheric aerobic counts on lettuce raw material and those on finished shredded lettuce product-several out breaks of *Salmonellasis* that were associated with cut cantaloupe and water melon have resulted from *Salmonella* present in the united states on the rind contaminated in the field or packing house (Harris *et al.*, 2003). Inoculation of *Listeria monocytogenes* and *Salmonella* on the surface of entire cantaloupe resulted in the contamination to fresh-cut pieces during cutting (Ukuk and Fett, 2002). These results indicate that bacterial on the surface of whole produce are that same as those on fresh-cut produce and can contaminate finished product through processing. Fresh-cut products can also be contaminated by spoilage microorganisms through contact by people or equipment during processing possibly by air during processing and packaging steps, especially in facilities that have been used for produce processing over an extended period of time. Cantwell and Suslow (2002) found significantly higher bacterial counts during processing on automated cutters and package fillers of a lettuce processing line, indicating that clean product can become relations where vegetables and fruit debris can accumulate, such as cutters and package-filling equipment. Shredding slicing steps in fresh-cut processing resulted in increased microbial population by 1-3 logs on cut cabbage, lettuce and onions (Garg *et al.,* 1990) and at least a 1-log increase for lettuce and chicory salads (Jockel and Otto, 1990).

Microbiological spoilage mechanisms in fresh-cut fruit and vegetable

Growth of microorganisms subsequently forming visible colonies is a common cause of spoilage of fresh-cut fruit and vegetables. There is in general a linear relationship between the microbial cell numbers and spoilage of cut produce during refrigerated storage (Zhuang *et al.,* 2003). This relationship stronger for fresh-cut melons and fruits compared with fresh-cut vegetables have been established for quality of fresh-cut produce-for example, in France and Germany, Microbiological specifications for mesophilic aerobic bacterial populations or aerobic plate counts (APC) of salad vegetables at production (fresh) are $5x10^{6}$ cfu/g, for separating good quality from marginally acceptable quality, and at use by date are $5x10^{7}$ cfu/g (Lund, 1993). However, the exceptions to this over all positive linear relationship have been reported in many studies (Zhuang *et al.,* 2003). Sappers *et al.* (2001) did not observe a consistent difference in total APC between unspoiled and spoiled cut cantaloupe during refrigerated storage. However, in the same report, a lower APC was consistently associated with unspoiled products. These phenomenon suggest the following.

1. Most, if not all, of the analyses that are currently conduction to determine microorganism populations of cut produce during storage are not specific enough to be associated with the shelf life or spoilage.
2. Low microbial counts are necessary to avoid or reduce spoilage of cut produce; however, high total microbial populations do not always correlate with spoilage.
3. It is not practical to use a microbiological specification based only on nonspecific microbial test results to reject fresh-cut products on a commercial level. APC and yeast, or lactic acid bacterial counts cannot be solely to Judge or predict shelf life or spoilage of lot of production although there is an overall linear relationship between microbial loads a quality of fresh-cut produce.

Formation of organic acids such as lactic acid and acetic associated with decreased *pH* values and generation of volatile compounds such as ethanol from fermentation of sugar by yeasts are additional mechanism that result in aroma and flavor defects of fresh-cut products stored or packaged under MAP. Bacterial soft rot, which is characterized by water-soaking and formation of a slimy surface on plant tissues, has been identified as the leading cause, of storage disorders in many types of whole produce and is frequently observed in fresh-cut fruits (Ukuk and Fett, 2002) and vegetables during storage. Bacterial soft rot result from degradation of plant cell walls by pectolytic enzymes produced by a variety of microorganisms, including *E. carotovora, P. marginalis, Batrytis, Clostridium, Alternaria, Geotrichum,* and *Fusarium* (Bulgarelli and Brackett, 1991). Many microbes use pectolytic enzymes to overcome plant

defense mechanisms and access plant nutrients. The pectin methyl esterase (PME), and polygalacturonase (PG), pectin lyase (PNL) and pectate lyase (PL), can degrade pectin's in the middle lamella of the cell, thereby resulting in liquefaction of the plant tissue leading to conditions such as soft rots other enzymes such as hemi-cellulose, cellulose, and proteases are also involved in the spoilage process but are usually secondary to decades, molecular genetic research has revealed that multiple isozymic forms of the pectolytic enzymes exist. These are inducible and are not equally involved in the degradation of plant tissues. Their importance in soft-rot formation is dependent on bacterial genus.
In summary, microbial spoilage of fresh-cut produce can be coursed by microorganisms or microbial growth processes. There is a strong relationship between microbial populations and shelf life if the cause of quality failure is visible growth of micro flora on the surface. However, there is a relationship between microbial cell numbers and shelf life if the cause of quality failure requires specific metabolic activity, particularly under conditions of temperature abuse.

Prevention and control of microbial spoilage

Many thermal and non thermal technologies have been developed to control microorganisms on fresh-cut produce. These have been summarized by Farber *et al,*. (2003). Types of thermal processing used to treat fresh-cut produce include hot water, hot steam, and hot sanitizing solution. Thermal processing is a relatively new technology to the fresh-cut produce industry. Laboratory studies with inoculated whole melons revealed that the thermo safe process can effectively reduce microbial cell numbers on the surface of produce by 5 log units. The process time (from seconds to minutes) and temperature (from 60 to 100^0C) are dependent upon the commodities being treated. There are a number of difficulties associated with the application of thermal processes to fresh-cut fruit and vegetables for example; thermal processed cannot be used for fresh-cut commodities such as deterioration of quality characteristics. Also, can other processed products be called fresh-cut after a thermal process treatment is applied? Another limitation is that thermal processes are generally an inefficient use of energy and create a challenge for cold chain management which is needed by fresh-cut processes for product distribution. Further research and development is needed to validate thermal processes that can achieve a 5-log microbial reduction without affecting the quality of processed produce. Non thermal technologies can be classified as either physical or chemical. Physical technologies include high pressure, irradiation, pulsed electric fields, pulsed white light, ultrasound, and ultrasound radiation. Some of these methods are generally not applicable commercially because they are too expensive (high pressure and pulsed electric fields), do not have consumer acceptance of efficacy (UV and pulsed white light). The mechanisms and application of these methods have been well reviewed by Lund *et al.* (2000) and Ohlsson and Bengtsson (2002).

Chemical technologies can be divided into gas-phase sanitation and liquid-phase sanitation based on the physical state of the chemical used. Examples of gas-phase sanitation include ozone and chlorine dioxide. One of the difficulties in the application of gas-phase technologies is that a special in-line closed system is needed for the treatment of produce. These applications could also pose an employee safety issue. The most widely used chemical treatment in the fresh-cut produce industry is chlorinated water.

In addition to these active control measures, other factors important in the prevention of microbial spoilage include raw material quality, processing technologies good manufacturing practice (GMP), packaging, and temperature management. High-quality raw materials can both reduce the potential for surface contamination and maximize the plant self-defense system. Diseased or damaged products are difficult to prevention and treatment and can contaminate products with low levels of microbes. Zhuang *et al.,* (2003) found that during storage yeast populations were significantly higher on cut honey dew melons having soft tissue than form melons that were firm. It is commonly known that a climacteric fruit, the increase in respiration just prior to full ripening generally coincides with a major reduction in fruit resistance to pathogens. Damaged cells have greater rates of subsequently leading to cellular senescence or death and increased susceptibility to fungal colonization.

Methods for Detection and isolation of organisms that causes spoilage on vegetable

The methods used to detect and isolate spoilage microorganisms are mainly based on cultural procedures. For example, for fresh-cut fruit O'Connor Shaw *et al.* (1994) extracted microorganisms from the fruits (1:5 dilution) using sterile 0.1% peptone water and 0.5% sodium chloride, macerated this preparation by stomaching for 1 min and using the following methods for enumerating different microorganisms: standard methods agar (SMA) with incubation at 25^oC for 3days for aerobic plate counts; dichloran rose Bengal chloramphenicol agar with incubation at 25^oC for 5days for yeasts and molds; Man, Ragusa and Sharpe (MRS) agar with anaerobic incubation at 30^oC for 6days for lactic acid bacteria. Ukuku and Fett (2002) enumerated microbes on cut melon (20g), using plate count agar (PCA) and incubation at 30^oC for 3days for mesophilic aerobic bacteria; PCA+ crystal violet at 30^oC for 3 days for Gram-negative bacteria; *Pseudomonas* isolation agar and incubated at 27^oC for 3days for *Pseudomonads;* MRS agar with 0.08% sorbic acid and incubation at 30^oC for 3days for lactic acid bacteria and (Zapek malt agar (CMA) for yeast and molds Allende *et al.,* (2002) enumerated microbes on mixed vegetable salads (30g) by plating homogenates in peptone saline on: PCA and incubating at 22^oC for 3days for total psychotropic bacteria; MRS agar and incubating at 30^oC for 3days for lactic acid bacteria; yeast glucose *chloramphenicol* agar and incubating at 30^oC for 3days for yeasts; violet red bile glucose agar and incubating at 37^oC for 2days for *Enterobactriaceae.* In the fresh-cut produce industry, 3m petrifilm methods are widely used to enumerate total plate counts, coli forms, lactic acid bacteria, and yeasts and molds because of convenience and minimal need for incubator and operating space. Specificities and detailed operating procedures of many of these methods are described by Downes and Ito (2001) and Sapers *et al.,* (2005).

Microbiological Spoilage Defects of Fresh-cut fruit and vegetables

Microbiological spoilage defects of fresh-cut fruit and vegetables include microbial colony formation or visible

microbial growth mainly due to microorganism proliferation, off-aroma and off-flavor formation mainly due to fermentation of sugar, soft-rot/water soak and sliminess due to enzymatic pectolyzation, and discoloration. For example, 0'connor-shaw *et al.,* (1994) observed mold growth at 14days on cut pineapples held at 4^{o}C and at 4 days when held at 20^{o}C. White mold colony formation was observed at 11days on cut cantaloupe held at 4^{o}C and at 7 days on cut honeydew heed at 8.5^{o}C. Visual evidence of bacterial spoilage of cut cantaloupes result from the presence of bacteria colonies, slime, and Juice turbidity and off odors (Sapers *et al.,* 2001). Mixed lettuce stored in bags with on oxygen transmission rate (OTR) of 15ml became in edible with 4days at 7^{o}C due to the off-odor an unacceptable taste that were described as alcoholic and fermented (Jacxsens *et al.,* 2003). Mixed bell peppers held at 7^{o}C were rejected by a trained sensory panel within 6days due to an acid odor and taste. Fresh-cut cantaloupe held at 4^{o}C developed on off-odor within 11days, and mixed vegetables salad held at 4^{o}C were spoiled by off-odors within 7days (Allende *et al.,* 2002). Shredded carrots packaged in a modified atmosphere and held at 10^{o}C developed off-flavors and became slimy.

Another very common defect of fresh-cut vegetable attributed to microbiological spoilage is water soak/soft rot. The decay of fresh-cut celery segments strode at < 5^{o}C in sealed film bays begins with water soaking at the cut surface and slimy moisture accumulation inside the bags. In a salads mix, endive and loleo blonde developed soft rotting more rapidly than the other components. Water soaking has been most commonly associated with spoilage of cut cantaloupe, honeydew, and water-melon (Ukuk and Fett, 2002). Especially under abusive storage temperature (>4^{o}C), although there is no direct evidence that this results from the activity of pectolytic bacteria .studies have revealed that spoilage microbes such as *Gluconobacter* can cause discoloration of whole produce, and fungal spoilage has discolored cut apples treated with antioxidants.

MATERIALS AND METHODS

Materials

All materials used in this work are of analytical grade. See attached on Appendix 1

Collection of Samples

A total of 6 samples of carrot, Tomatoes, green beans, cabbages, cucumber and Onions were collected in a polythene bag from Eke Awka market in Awka South Local Government Area of Anambra state, Nigeria. The samples was taken to the laboratory for analysis.

Isolation of Bacteria by Spread Plate method

Bacteria counts were determined after imposing main and sub treatments. Main treatments were washed with ordinary tap water and washing with sterile distilled water. The sub treatment were peeling of the outer covering. The salad samples were put in a different conical flask containing sterile distilled water and placed on shaker for 10 minutes to maintain a uniform mixture. Now, the isolation was carried out using ten fold serial dilution and spread on nutrient agar, Macconkey agar plate.

Purification of isolates

Isolates obtained were purified by sub culturing onto sterile nutrient agar plates.

Identification of isolates

Isolates were characterized on the basis of morphological analysis, biochemical test and Gram's reaction. Catalase test, motility test, coagulase test, Indole test, spore test, methylred test, Indole test, spore test, citrate test, voges proskauer test, citrate test, glucose test, lactose test, maltose test and mannitol test as was done by Cruckshank *et al.,* (1986). The isolates were identified according to the scheme of Holt *et al.,* (1984).

GRAM STAINING

This is the most important widely used procedure for characterizing bacteria. It was first described by Christian Gram. This method divides the bacteria into two groups, Gram positive which is purple in colour and Gram negative which is pink in colour. This technique is based on the ability of bacteria to retain primary stain (crystal violet dye) during decolourisation with alcohol or acetone . Gram positive bacteria retain primary stain while Gram negative bacteria are decolourised by alcohol and takes up the red colour counter stain. A smear of an isolate was made on a clean slide and allowed to dry. It was then heat fixed by passing the smear through the bursen burner, this is done to enhance the sticking of the organism on the microscope slide. The smear was flooded with crystal violet and left for 60sec before washing off with water . Lugols iodine was added and allowed to stand for 60 sec before being washed off and decolourised with alcohol for 10 secs. The slide was then washed off, stained with safranin for 30 sec washed off and allowed to air dry . A drop of immersion oil was added to the slide which was then viewed under the microscope using the x 100 objective lens.

MOTILITY TEST

Each bacteria isolated was separately inoculated into a semi-solid medium using sterile straight wire and incubated at 37^{O}C for 24h. Migration of the isolates away from the line of inoculation was a positive result while lack of migration away from the line of inoculation indicated a negative result.

CATALASE TEST

This test is used to detect the enzyme catalase which protects the bacteria from hydrogen peroxide accumulated which can occur during aerobic metabolism. Catalase breaks the hydrogen peroxide into oxygen and water. The organism was picked and emulsified on a clean slide. A drop of 3% hydrogen peroxide was added to the slide. The presence of sustained bubbles indicated a positive result while their absence indicated a negative result.

COAGULASE TEST

This is used to distinguish pathogenic Staphylococus aureus which produces the enzymes coagulase from Streptococcus which do not produce coagulase. Coagulase causes serum to clot by converting fibrinogen to fibrin. One milliliter plasma was dropped on a clean slide and the organism emulsified in it. The dumping of the organism within 0 seconds indicates a positive result.

CABOHYDRATE FERMENTATION TEST

This test is used to detect organism which utilize different sugar as sources of energy with the production of acid and or gas. The sugars used were glucose,maltose and lactose. Peptone water broth was prepared. Bromothymol blue indicator was added to the broth in three separate conical flasks containing glucose, maltose and lactose. The above solution (peptone water broth + indicator) was added at equal proportions. Five milliliters of the mixture was then dispensed into Bijou bottles. Durham tubes were added and the bottles were sterilized in the autoclave at 121^{o}C for 15 min. After cooling, the test organism was inoculated into each of the Bijou bottle and inoculated for 24h. Acid production was indicated by a change in colour of the mixture while gas production was indicated by bubbles in the Durham tubes.

INDOLE TEST

This test was carried out to determine the organism that break down the amino acid tryptophan into indole. The test organism was incubated in sterile test tubes containing peptone water and incubated at 37^{o}C for 48h. 0.5ml of kovac reagent was added and mixed to stand for 10 min. The development of a pink color indicated a positive result.

SPORE TEST

Bacteria film was made on a slide and heat fixed with minimal flaming. The slide was placed in the rim of a beaker of boiling water, with the bacteria film upper most. The film was flooded with a 0.05% aqueous solution of malachite green when large droplet have been condensed on the underside of the slide and left to act for 60 sec while the water continue to boil. The slide was washed in cold water and treated with 0.5% safranin solution and left for 30 sec. The slide was washed with clean water, dried and viewed under the microscope. This method colored the spore green and the negative bacillia red

METHYL RED TEST

The test organism was introduced into glucose phosphate peptone water and incubated at 37^{o}C for 48h. 5drops of methyl red reagents were added, mixed and the result read. A red coloration indicated a positive result while a yellow coloration indicated a negative result.

CITRATE UTILIZATION TEST

The medium used was simmone citrate agar. The test is used to identify each organism which of the organism can utilize citrate as the sole source of carbon for metabolism. It is used in the differentiation of the organism in the enterobacteriaceae and other genera. Bijou bottles were used for the test in saline preparation of the organism was inoculated inside the citrate medium and incubated at 37^{o}C for 24h. A change in color from green to blue indicates a positive result

VOGES PROSKAEUR TEST

The test organism was introduced into glucose phosphate. Peptone water was incubated at 37^{o}C at 48h. 5 drops of Barrites A (alpha napthol) and Barrite B (potassium hydroxide) reagent were added, mixed and the result read. A pink burgundy coloration indicated a positive result

Total viable count

One millimeter each of the enriched samples was serially diluted and 0.1ml aliquot of the serially diluted sample (10^{7}) was introduced into sterile plates and sterile nutrient agar added. Duplicates plates were prepared. Ketoconazole was introduced at a concentration of 0.05mg/ml to inhibit fungal growth. Incubation was carried out in an inverted position at 30^{o}C for 24h after which the bacteria colonies that developed were counted and result recorded. Each colony was sub cultured and stored in sterile nutrient agar for characterization and identification.

RESULTS

In general, the bacteria lead on the salad vegetable varied. The total viable count ranges from 1.83 x 10^{7}cfu/g to 3.26 x 10^{7}. Carrot had a total viable count of 3.26 x 10^{7} and it is the vegetable with the highest bacteria load.

Tomatoes had 2.43cfu/g, green beans had 1.83cgu/g, cabbage had 2.93cfu/g, cucumber had 2.83cfu/g and onions had 2.43cfucfu/g. all the salad sample had microorganism in them. Some had gram positive while some had gram negative bacterial organisms. The morphology and biochemical characteristics of the bacteria isolates from the salad samples were shown in Table 2. The organisms were characterized and identified as the species of *Staphylococcus, Bacillus, Salmonella, Esherichia coli, Pseudomonas* and *Staphylococcus aureus.*

Table 1: Total viable count of the salad vegetable sample

Sample	Type of sample	Total viable count (cfu/g)
1	Carrot	3.26 x 10^{7}
2	Tomatoes	2. 43 x 10^{7}
3	Green beans	1.83 x 10^{7}
4	Cabbage	2.93 x 10^{7}
5	Cucumber	2.83 x 10^{7}
6	Onions	2.43 x 10^{7}

Table 2: Morphology and Biochemical characteristics of the Isolate from Carrot, Tomatoes, Cabbage , Green beans, Cucmber, Onions

Sample	Isolate	Form	Gram reaction	Motility test	Catalase test	Coagulase test	Indole test	Spore test	Methyl red test	Voges praskaeur test	Citrate utilization test	Glucose	Lactose	
Carrot	*Staphylococcus sp*	Coccus in cluster	+	-	+	-	-	+	-	+	+	+	+	+
Tomatoes	*Bacillus sp*	Single rod	+	+	+	+	+	-	+	-	-	+	+	+
Cabbage	*E. coli*	Blue cluster rod	-	+	+	-	+	-	+	-	-	+	+	+
Green beans	*Salmonella sp*	Short rod	-	+	+	-	-	-	+	-	-	+	-	+
Cucumber	*Pseudomonas sp*	Single cocci	-	+	+	+	-	+	+	-	+	+	+	+
Onions	*Staphylococcus sp*	Cocci in cluster	+	-	+	-	-	+	-	-	+	+	+	+

+ = Positive result

- = Negative result

III. DISCUSSION

Vegetables that are used as salad have been implicated as a cause of food poisoning and thus, they are hazardous to the health of the consumers who are infected with many types of disease. This could be linked to the fact that most of these vegetables are consumed without being subjected to thorough washing (Lund, 1992). The result obtained from this research work showed that there is a lot of bacteria count on these salad vegetables. From the result that was got on total viable count of 3.26×10^7, Tomatoes has 2.43×10^7, green beans has a viable count of 1.83×10^7, cabbage has 2.93×10^7, cucumber has 2.83×10^7 and onions has 2.43×10^7. Carrot had the highest bacteria count and has higher than the total viable count of samples that was analyzed and reported by Uzeh *et al.,* ((2009). Carrots are usually harvested from the soil and these will result to it been contaminated with soil pathogens. Tomatoes has bacteria pathogens in it and the organism that was isolated was *Bacillus*, a gram positive organism. Cabbage has *E. coli* in it and the bacteria load is high. Green beans has a low bacteria lead and the organism isolated from it was *Salmonella* while cucumber and onions was analyzed and *Pseudomonas* and *Staphylococcus* was found in the salad vegetables. The result got in this research work agreed with the analysis done by Abdullahi and Abdulkareem, (2010).

Contamination of these organism might arise from washing the vegetables with contaminated water or handling by infected marketer. The presence of these organisms can cause food borne diseases in consumers of these product.

IV. CONCLUSION

Carrot has the highest bacteria count while green beans has the lowest bacteria count. These bacteria organisms can serve as an indicator for the need to promote awareness about the possible health hazard that could be due to poor handling of these vegetables. Therefore, there is the need for regulation bodies to ensure that microbiological standards are established and practiced by farmers and marketers for the handling and distribution of salad vegetables.

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APPENDIX

Materials needed

Nutrient agar
MacConkey agar
MRS agar
Petri dish
Distilled water
Conical flask
Syringes
Masking tape
Test tubes
Wire loop
Glass slides
Weighing balance
Autoclave
Bunsen burner
Hydrogen peroxide

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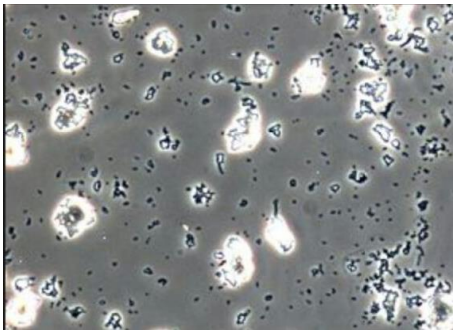
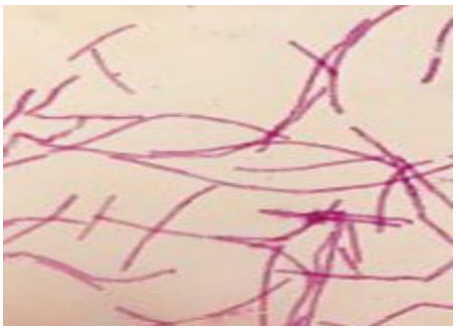
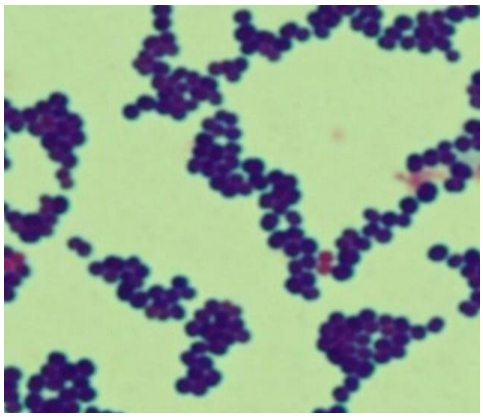
MICROSCOPIC VIEW OF BACTERIA FOUND IN SALAD VEGETABLE



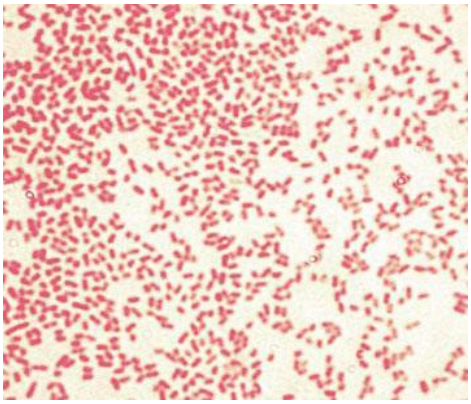
E. Coli



Bacillus anthracis



Staphylococcus aureuss



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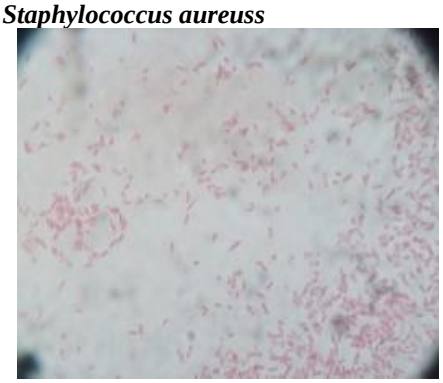
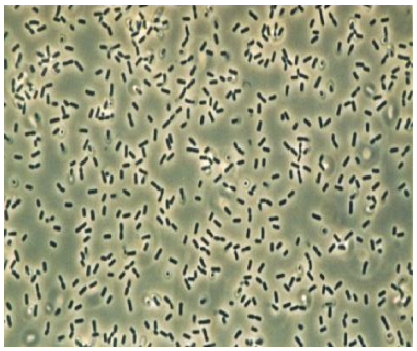


Salmonella typhis



Bacillus subtilis