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Bacterial Burden of Vegetables Salad Sold in Some Fast Food Centers in Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The demand for vegetables salad is high because it contains essential ingredients good for health. Its importance in diets is advocated by nutritionists but the consumer may be exposed to risk such as infection. Pathogens may contaminate raw vegetables and cause food borne illness or outbreak.
Aim: The aim of this work is to isolate and identify bacteria associated with vegetables salad in Port Harcourt.
Methodology: A total of 100 samples of ready to eat vegetables salad were examined, ten (10) samples were purchased from each fast food centre. Out of the 10 samples, five (5) were purchased in the morning, while 5 were purchased in the afternoon. In a wide mouth sterile glass container, 10g of vegetables salad were added to 90mL of prepared sterile normal saline and subsequent

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serial dilution were made by adding 1ml to 9ml of diluents to 10^3 and 0.1ml of last dilutions were plated by spread plate technique on both nutrient and MacConkey agar. The plates were incubated 37^0C for 18-24 hours and examined for growth.

Results: The bacteria isolated from vegetables salad were *Staphylococcus aureus* (45%), *Escherichia coli* (36%) and *Bacillus sp* (18%) respectively. The percentage occurrences of bacteria isolated in morning were 35.6% whereas the isolates in afternoon were 64.4%. The viable count from the vegetables salad ranged from $2x10^3$ to $6.5 x10^3$ cfu/mL

Conclusion: Vegetables meant for preparation of vegetables salad should be properly washed with good water and handled hygienically to avoid contamination. Prepared vegetables salad should be consumed shortly after preparation to avoid multiplication of contaminating bacteria.

Keywords: Bacteria; contamination; vegetables salad; food centers; Port Harcourt.

1. INTRODUCTION

Raw vegetables are necessary ingredients for health, this has made the demand for vegetables to rise. Nutritionists have emphasised the importance of raw vegetables in diets, and the consumption of fruits and vegetables are recommended daily [1] Vegetables contain carbohydrates, anti-oxidants, minerals, vitamins and fibers and some of these nutrients are heat labile [2]. Vegetables have health promoting properties such as phytoestrogen and anti-inflammatory agents [3,4]. Lack or inadequate fruits and vegetables in diet may contribute to poor health and cause condition like non-communicable disease [5]. Vegetables as part of diet contribute to weight loss and reduce obesity [6]. Because of the health benefits of vegetables there was global promotion on consumption of fruits and vegetables [7,8]. Vegetables were consumed raw as salad to retain the natural taste and heat labile nutrients [8]. Aside the health benefits of vegetables, the consumption of fresh vegetables may be associated with consumer risk [9]. Human infections linked to the consumption of raw fruits and vegetables are on increase [8]. Vegetables had been pin pointed as vehicle that can transmit bacteria associated with food-borne illness [10]. Increase in the number of immune-compromised consumers might enhance the rate of infection [8]. Many reports have specified that pathogenic bacteria may attach to the surfaces of fresh vegetables and their consumption may cause food-borne infection or outbreak [11]. Contamination may also occur by use of dirty water and cross-contamination by infected food-handlers [12]. The contamination of vegetables may occur before harvest when contaminated manure, sewage, irrigation water, wild and domestic animals, or during harvest, transport, processing, distribution, marketing and storage [10,13,11]. Bacteria frequently isolated from the surfaces of vegetables are mostly Gram-negative saprophytes which may survive beyond washing, therefore precautionary measures must be taken since they may form biofilm on the vegetables surfaces or might be protected in vegetables cuticles [14]. Food-borne illness associated with *E. coli* is important and may be of public health attention. The presence of *E. coli* might suggest feacal contamination. The outbreak of *E. coli* O157:H7 due to the consumption of contaminated spinach led to the hospitalization of 200 patients and 3 cases of death [15]. Foodstuffs may also serve as medium of transmitting bacteria especially when eaten raw [16,17,18]. The presence of *E. coli* which may imply feacal contamination might as well be a pointer to the possible presence of other enteric pathogens such as *Salmonella, Shigella* and pathogenic strains of *E. coli* [19,20,21,22]. It had been reported that 69% of salad samples served in restaurants in Ilam, Iran, were contaminated with *E. coli* [23]. The safety of vegetables eaten raw might be of concern because they may harbour bacteria that may be pathogenic [14,24] and parasites [25,26]. Poor hygienic practices and post-harvest handling may aid contamination [15,26]. There are documented outbreaks of human infections caused by the consumption of raw vegetables [27]. Trade has also contributed to geographic spread of these bacteria [28]. Researches in developed and developing countries have shown the potential role the consumption of raw vegetables play in transmitting pathogenic microbes [29,30,31,32]. Other investigators have isolated bacteria such as *S. aureus, Enterobacter* sp.*, Klebsiella* sp.*, Salmonella Typhi, Serratia* sp., *Providencia sp.*, *Pseudomonas aeruginosa, Yersinia enterocolitica, Aeromonas hydrophila* and *Shigella sonnei* [31] and *E. coli*, *Pseudomonas aeruginosa*, *Bacillus sp, Enterobacter sp.* and *Proteus sp.* from vegetables salad [32].

Owing to awareness created on the health benefits of vegetables and its advocated consumption, the aim of this study is to determine the bacteriological quality of vegetables salad sold in Port Harcourt and identify the potential pathogens that may be associated with it.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Port Harcourt, Rivers State, Nigeria in the month of November 2017. Port Harcourt is the capital of Rivers State. It is situated on latitude 4.75ºN and longitude 7º E, located in the Niger Delta Region. Port Harcourt is in tropical rain forest with lengthy wet season and heavy rainfall with short dry season. The temperature all through the year is relatively constant (25-28ºC). The major occupation of the people are fishing and farming.

2.2 Collection of Samples

Samples of ready to eat salad were purchased from ten (10) different fast food centers located in Government Reserved Area (GRA). The choice of GRA was because most fast food centers were located there. The food centers were designated A to J. A total of 100 samples of vegetables salad were examined, ten (10) samples were purchased from each fast food centre. Out of the 10 samples, five (5) were purchased in the morning (about 10.00 am) while 5 were purchased in the afternoon (about 2.00 pm). The sample size enables collection of the samples in about an hour, considering distance and traffic jam in Port Harcourt. The samples were transported to the laboratory in a cooler of icebergs for bacteriological examinations.

2.3 Cultivation of Samples (Bacteriological Examinations)

In a sterile wide mouth glass container, 10g of purchased vegetables salad sample was added to 90 mL (neat 90 mL of prepared sterile normal saline) and dilution made up to 10^3 (water) by subsequently transferring 1mL of the homogenate to 9mL sterile normal saline in fresh test tube. Then 0.1 ml of the last dilution 10^3 was plated by spread plate in duplicate with the aid of a sterile glass rod on nutrient and MacConkey agar. The plates were incubated at 37ºC for 18 – 24 hours and examined for growth.

2.4 Identification of Isolated Bacteria

Multiple tests including colonial appearance, chemical and biochemical tests were used for the identification of isolates such as: Gram's stain, motility, catalase, coagulase, indole, citrate, oxidase, methyl red, carbohydrate fermentations [33].

3. RESULTS

3.1 Percentage Occurrences of Isolated Bacteria

The bacteria isolated from ready to eat vegetables salad from the fast food centers were *S. aureus* 82(45%), *E. coli* 64(36%) and *Bacillus sp* 34(19%) respectively as shown in Fig. 1.

3.2 Percentage of Isolated Bacteria from Each Fast-food Centre

The percentage occurrences of isolated bacteria from each fast food centers were A, 27(15%), B, 22(12.2%), C, 16(8.8%), D, 12(6.7%), E, 11(6.1%) F, 17(9.4%), G, 17(9.4%), H,18 (10%) I,19 (10.6%) and T, 21 (11.7%) respectively. The highest count were obtained from fast food center A,15% and the least from E, 6.1% as shown in Fig. 2.

3.3 Percentages of Isolated Bacteria from Different Fast Food Centers

The percentage occurrences of *S. aureus* from the ten fast food centers were from 9 (33.3%) in fast food A to 7 (63.6%) in fast food E. *E. coli* ranged from 2 (16.7%) in fast food center D to 9 (47.9%) in fast food center I, while *Bacillus sp* was from 1 (5.3%) in fast food I to 9 (33.3%) in fast food center A respectively. *S. aureus* were the most prevalent in nine (9 food centers, Fig. 3).

3.4 Percentages of Isolated Bacteria from Different Fast Food Centers in the Morning and Afternoon

The percentages of bacteria isolated in morning (about 10.00 am) were 64 (35.6%) whereas the isolates in the afternoon (about 2.00 pm) were 116 (64.4%) respectively (Fig. 5).

3.5 Comparison of Isolated Bacteria Obtained in Morning and Afternoon from the Fast food Centers

The percentages of bacteria isolated from each fast food centers in the morning were as follows:

Morning A, 9(33.3%), B, 6(27,3%), C, 6(37.5%), D, 4(33.3%), E, 4(36.4%), F, 7(41,2%), G, 7(41.2%), H, 6(33.3%), I, 7(36.8%) and J, 8(38.1%) respectively. The percentage of bacteria isolated from the fast food centers in afternoon were: A, 18(66.7%), B,16(72.7%)), C, 10(62.5%), D, 8(66.6%), E, 7(63.6%), F,10(58.8%), G, 10(58.8%), H, 12(66.6), I, 12(63.2%) and J, 13(61.9%) respectively. The values in the afternoon were twice the values obtained in morning.

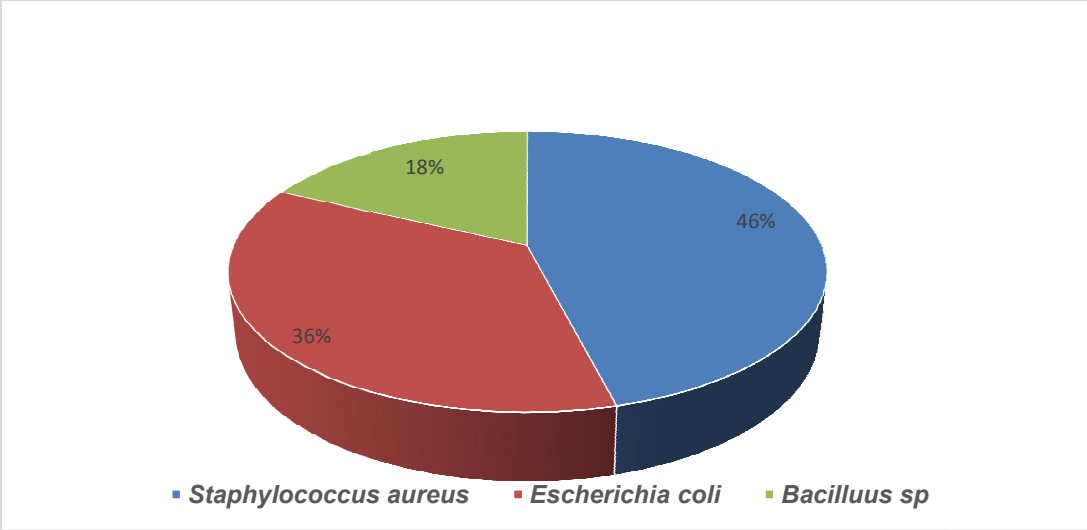

Fig. 1a. Percentage Occurrences of Isolated Bacteria

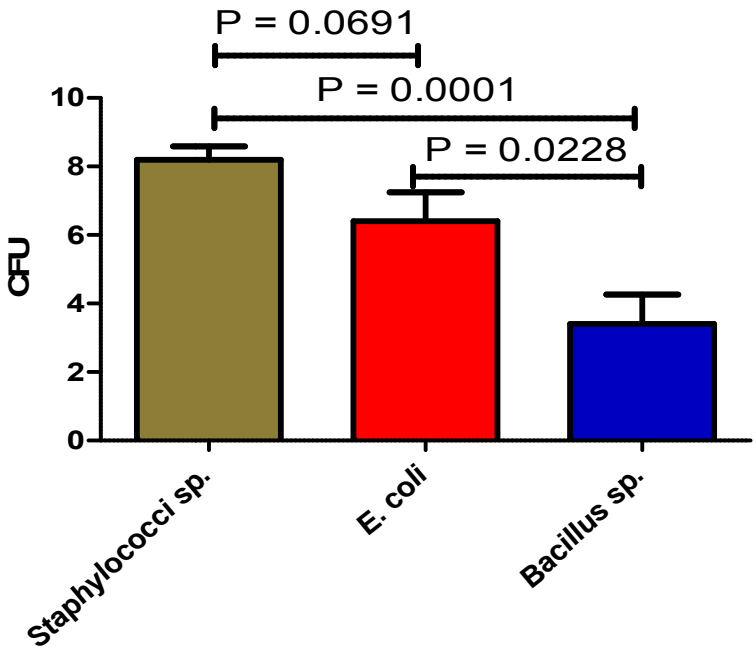

Fig. 1b. Mean values of isolated bacteria

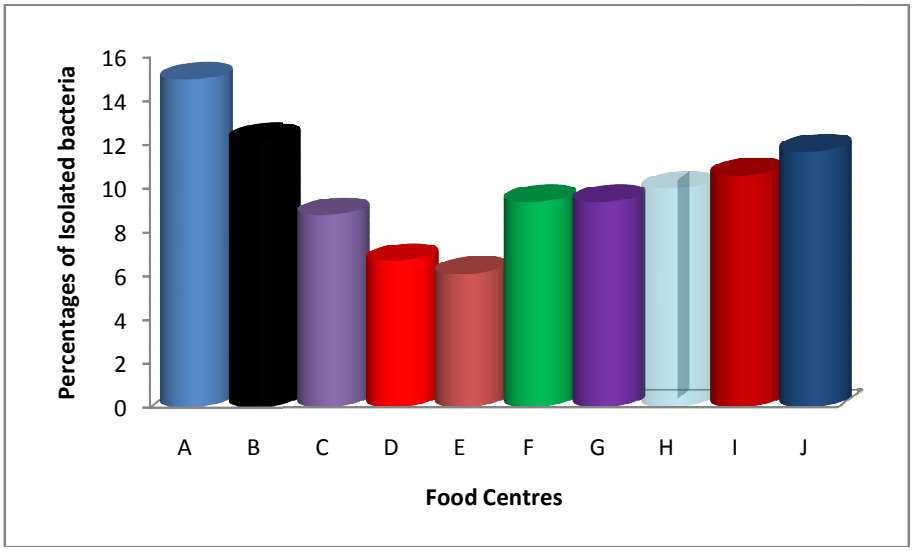

Fig. 2. Total percentage of bacteria isolated from each fast food centre

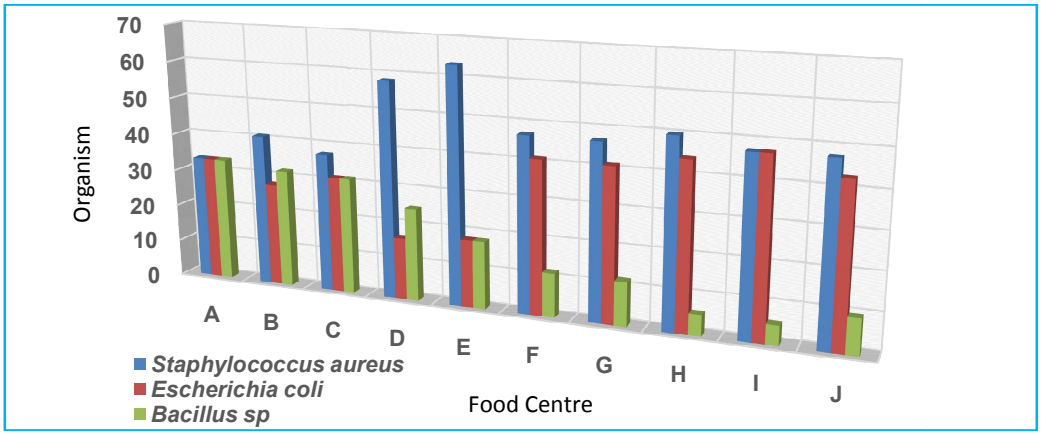

Fig. 3. Percentages of isolated bacteria from different fast food centers

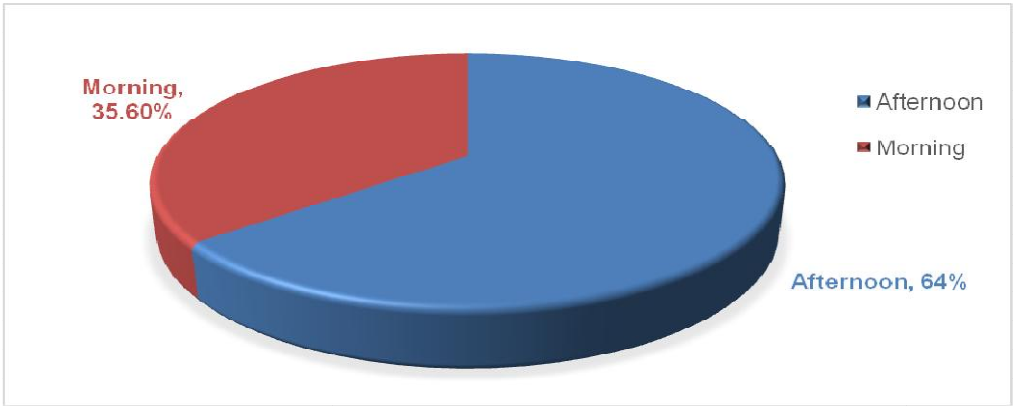

Fig. 4a. Percentages of isolated bacteria from different fast food centers in the morning and afternoon

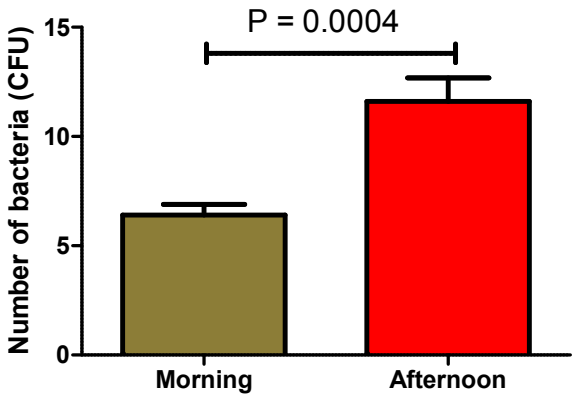

Fig. 4b. Mean values of isolated bacteria in morning and afternoon

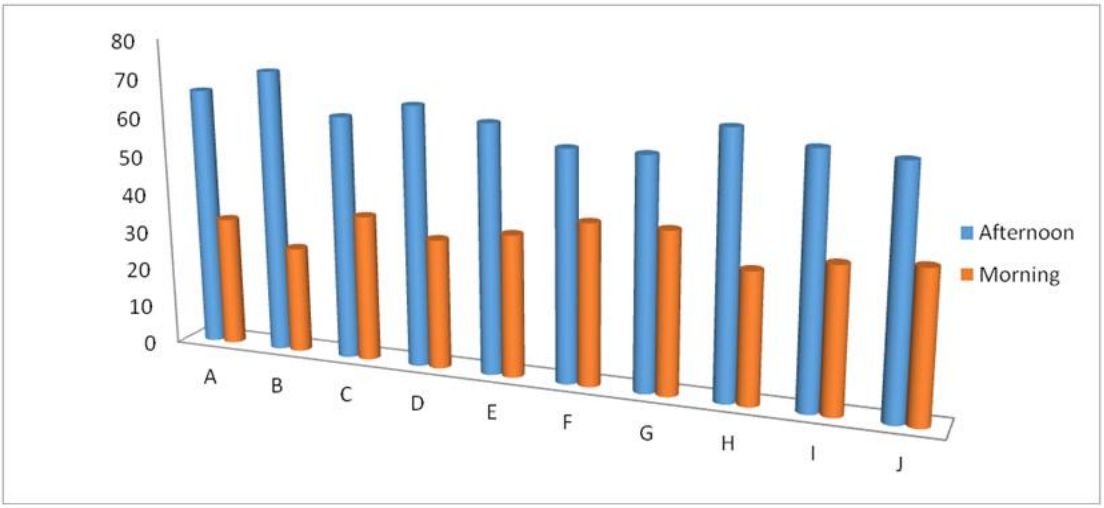

Fig. 5. Comparison of bacteria isolated from vegetables salad bought in morning and afternoon from the fast foods centers.

4. DISCUSSION

The bacteria isolated from vegetables salad were *S. aureus*, *E. coli* and *Bacillus species.* The results were in line with that of other workers [34, 35] who reported these bacteria as commonly isolated organisms from vegetables and vegetables salad. World Health Organization also listed these organisms among the organisms that may be associated with food borne illness [36]. *S. aureus* were the highest in percentage prevalence 46%, followed by *E. coli,* 36% and *Bacillus sp* 18% respectively The bacteria counts obtained from the samples examined may reflect the rate at which vegetables might be contaminated with bacteria which were also observed by other researchers [8,10,13]. It might be necessary to consider possible critical control points from harvest to preparation of vegetables salad for consumption and also educate the workers on the principles of food preservation and hygiene to avert risk factors that might aid contamination. Some of these bacteria may be carried over from previous operations on utensils, equipment and water used in processing or preparation [8,9]. The isolation of *E. coli* may indicate faecal contamination with human or animal faeces or excessive human handling. Another potential source of contamination could be from the water used in growing and processing vegetables before sale. *E. coli* were common isolated from vegetables salad [31]. *E. coli* is a natural inhabitant of the human gastrointestinal tract

Table 1. Identification of isolated bacteria

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H2S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fementative	Bacteria
1	M	R	E	C	D	+	Md	Rd	+	-	+	-	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	***Bacillus sp.***
7	Cr	S	E	C	Mt	-	Md	Rd	+	-	-	-	-	+	-	+	-	-	-	AG	AG	AG	AG	AG	AG	AG	+	AG	***E. coli***
4	Cr	S	E	C	Mt	+	Md	Co	-	+	+	-	-	-	-	+	N	N	-	A	A	A	-	A	A	-	+	+	***S. aureus***

Key: M =Milky, Cr =Creamy, R =Rough, S =Smooth, E =Entire, C =Clear, D =Dry, Mt =Moist, Md = Moderate, Co =Cocci, Rd =Rod, N = Not determined.

(GIT) but might occasionally be associated with disease in human. *E. coli* may also be present in soil and taint vegetables that are in contact with the soil. The presence of bacteria in vegetables salad might be linked to unhygienic practices by workers and the use of contaminated water in processing [1]. The presence of *E. coli* also may suggest the probable presence of other enteric pathogens that may be associated with gastroenteritis and might be transmitted through faeces such as *Salmonella sp*, *Shigella sp* and pathogenic strains of *E. coli*. The outbreak of *E. coli* O157:H7 due to consumption of contaminated spinach resulted in hospitalization of 200 patients and 3 cases of death, also the outbreak of food-borne disease which occurred in Europe and Germany in 2011 was mainly due to contamination of vegetables salad by harmful strain of *E. coli* O104:H4 [15,35,37]. Using t – test at $p< 0.05$ there was no significant difference in the numbers of *S. aureus* and *E. coli* isolated from vegetables salad.

S. aureus have been shown as the most prominent aetiologic agent of pyogenic infections and staphylococcal infections worsen most already existing superficial infections. *S. aureus* is a normal flora of humans, 25% of healthy individuals are carriers of this bacterium on their palms, nostril, skin etc. The percentage occurrence of *S. aureus* 46% may be linked with carriers or infected food handlers [34]. Studies conducted both in Nigeria and Cameroun have shown that *S. aureus* were the most predominant bacterium isolated from vegetables [32,34,35]. Other possible factors were temperature abuse by some fast food operators and reprehensible handling during preparations, sales and services, which may increase the chances of contamination and proliferation of bacteria, particularly in a country such as Nigeria which has unstable power supply [16,25]. There were similar findings by other researchers that vegetables salad food borne outbreaks due to *Staphylococci* could occur under conditions that favours the growth [29,30]. *S. aureus* in known to cause food poison (intoxication) and infection. There was significant difference between the numbers of *S. aureus* and *Bacillus sp*. isolated from vegetables salad using t- test at $p< 0.05$.

Bacillus sp were 18% of isolated bacteria. *Bacillus* can cause food poison and infection, which is attributed to its toxin producing ability and spore formation knack. The production of spores enables the bacterium to withstand unfavourable conditions such as low temperature as obtained in the refrigeration of vegetables salad. The fluctuation in temperature of most refrigerators because of unstable power supply may aid germination of spores and subsequent multiplication of the bacterium. The spores of *Bacillus sp.* are mostly geophilic, hence contamination of vegetables with the bacterium may occur when vegetables are in contact with the soil, coupled with other predisposing environmental factors. *Bacillus sp* were mostly isolated from cabbage and onion. *Bacillus* is responsible for various infections in human such as enteric infection associated with ingestion of contaminated foods and vegetables salad which may cause severe form of septicemia. There was also significant difference between the counts of *E. coli* and *Bacillus sp*. isolated using t- test at $p< 0.05$.

The bacterial counts from vegetables salad bought in afternoon (about 2.00 pm) were higher than that from vegetables salad bought in the morning (about 10.00 am).This observation might suggest that the vegetables salad were stored at holding temperatures that favoured the multiplication of the bacteria isolated from vegetables salad. Hydrogen ion potential (pH) and storage temperature are the two principal determinants of growth for food-borne pathogens associated with fresh produce. Only 20% of the fast food centers met required standard of 100 to 10^3 *E. coli* in ready to eat precut fruits and vegetables [38].

4. CONCLUSION

Vegetables salad preparation should be carried out with good water and handled hygienically to reduce contamination with bacteria such as *S. aureus, E. coli and Bacillus sp* that may cause food infection and poisoning. Education and training of food handlers on measures that will prevent contamination of vegetables salad should be advocated. Prepared vegetables salad should be consumed shortly after preparation to avoid multiplication of contaminating bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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