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https://dx.doi.org/10.4314/nvj.v39i4.5 **ORIGINAL ARTICLE**

Coliform Count and Isolation of *Escherichia coli* in Fresh Fruits and Vegetables sold at Retail Outlets in Samaru, Kaduna State, Nigeria.

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SUMMARY

Vegetables and fruits are commonly viewed as potential risk factors for infection with enteropathogens. One hundred and eight vegetables and fruits sold within Samaru, Zaria were cultured for coliforms and *Escherichia coli* using total aerobic plate and coliform counts on Nutrient and MacConkey agar plates respectively, and Eosin Methylene Blue for *Escherichia coli* isolation. The mean total aerobic plate count (TAPC) ranged between 1.05×10^{11} to 1.64×10^{11}. The highest TAPC (1.64×10^{11}) was in pineapple while the lowest count (1.05×10^{11}) was in water melon. There was no much difference between the TAPC of lettuce (1.44×10^{11}) and cabbage (1.42×10^{11}) as well as carrot (1.37×10^{11}) and cucumber (1.39×10^{11}). Samaru market had the highest (1.56×10^{11}) TAPC while Suleiman market had the least (1.30×10^{11}). The mean coliform count (TCC) ranged from 1.28×10^9 to 3.55×10^9 CFU/ml. Carrot (1.28×10^9) had the lowest TCC while cucumber (3.55×10^9) had the highest. Others were pineapple (2.02×10^9), water melon (3.05×10^9), lettuce (3.17×10^9) and cabbage (2.72×10^9) CFU/ml. Out of 108 samples obtained, 50 (46.3%) were suspected to be *E. coli* with 23 (46%) yielding reactions typical of *E. coli*, with an overall prevalence of 21.3%. Cucumber had the highest isolation of 5 (27.8%) while the least was water melon 1 (5.6%). Other coliforms isolated were *Klebsiella* (8.3%), *Enterobacter* (7.4%), *Citrobacter* (5.5%), *Proteus* (2.7%) and *Serratia* (0.93%) spp. This study has demonstrated the public health significance of consuming fresh fruits and vegetables sold in the study area as they may be sources of infection to consumers especially if the produces are not properly washed or cooked.

Key words: Coliform, *E.coli*, Fruits, Vegetables

INTRODUCTION

Vegetables are well recognized as important part of a nutritious and healthy diet (Berger *et al*., 2010). The consumption of ready to eat vegetables in Nigeria has greatly improved based on their proven medical and nutritional benefits (Adeshina *et al*., 2012). Vegetable is the edible part(s) of a plant, such as the stems and stale (celery), root (carrot), tuber (potatoes), bulb (onion), leaves (spinach, lettuce), flower (globe artichoke), fruits (apple, cucumber, pumpkin, strawberries, tomato) or seeds (beans, peas) (Gupta *et al*., 2003). Most vegetables have low calorie, saturated fat, sodium content and are devoid of cholesterol, they also contain vitamins and minerals such as ascorbic acid, folic acid, iron, zinc, magnesium, potassium, calcium, carotenoid and flavonoids (Ranganna *et al*., 1983; Gupta *et al*., 2003). Outbreaks of food infections associated with consumption of ready-to-eat vegetables have been increasing (Weldezgina and Muleta, 2016). Garg *et al*. (2010) showed that outbreaks of illness caused by bacteria, viruses, and parasites have been linked epidemiologically to the consumption of a wide range of vegetables and to a lesser extent of fruits. Consumption of vegetable product is commonly viewed as a potential risk factor for infection with enteropathogens such as *Salmonella* and *Escherichia coli*, presence of *E. coli* is an indicator of faecal contamination (Hanson *et al*., 2012).

Contamination of vegetables by coliforms is attributed to the wide distribution of coliforms in nature (Yabaya *et al*., 2012). Routes of contamination are varied and include application of organic wastes to agricultural land as fertilizers, contamination of waters used for irrigation with faecal materials, direct contamination by livestock, wild animals, birds, and post-harvest issues such as workers hygiene, transport containers, harvesting equipment's (Heaton and Jones 2008). Little is known about microbial ecosystems on the surface of raw vegetables, the pH of most vegetables is 4.6 or higher which is suitable for the growth of pathogenic bacteria (Carlin, 1994; Beuchat. 2002).

Vegetables can become contaminated with pathogenic microorganisms during harvesting through faecal material, human handling, harvesting equipment, transport containers, wild and domestic animals, air, transport vehicles, ice or water (Beuchat, 1995). Post-harvest treatment of fruits and vegetables include handling, storage, transportation and cleaning, environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption, poor handling can damage fresh produce, rendering the product susceptible to the growth/survival of spoilage and pathogenic microorganism and this damage can occur during packaging and transport (Francis *et al*., 1999).

Consumption of raw or minimally processed vegetables has result in diseased condition as studies have revealed that outbreaks are associated with etiologic agent of bacterial origin predominantly *Salmonella*, *Escherichia coli*, *Shigella* (enteric pathogens), *Listeria*, *Staphylococcus*, *Campylobacter*, *Vibrio* (Burnett and Beuchat 2001; Olaimat and Holley, 2012). Many infection outbreaks have been associated with water or food directly or indirectly contaminated by animal manure by identifying *Escherichia coli* and fecal coliforms, which are indicators of fecal pollution (Seraphin *et al*., 2016). The lack of an effective antimicrobial treatment at any step from planting to consumption means that pathogens introduced at any point maybe present on the final product. Washing and rinsing some types of fruits and vegetables prolongs shelf life by reducing the number of microorganism on the surfaces, however only a portion of pathogenic microorganism maybe removed with this simple treatment (Beuchat, 1998; Matthews, 2014a&b).

Fruits and vegetables are abundant in the study area, and these are handled unhygienically by the retailers as they sprinkle dirty water on these produces. Consumers consume these produces either raw, improperly cooked or improperly washed. Hence, this study was aimed at evaluating the coliform count of fruits and vegetables commonly sold within Samaru, Zaria to ascertain their fitness for consumption.

MATERIALS AND METHODS

Study Area

The study was carried out in Zaria LGA of Kaduna State. Zaria is located between longitude 11004'N and latitude 7042'E with an altitude of 550 – 700 meters above sea level and has a total land area of 300 Kmsq. Zaria is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7ºC and an annual rainfall of 1092.8mm 41, It is a medium sized city with an estimated population of 547,000. (Agbogu *et al.*, 2006). Zaria operates on the WAT time Zone. Zaria economy is primarily based on agriculture. Zaria is home to Ahmadu Bello University in Nigeria.

Sample Collection

In this study, two types of fruits (watermelon, pineapples) and four types of vegetables (lettuce, carrot, cabbage and cucumber) were selected for the analysis. The fruits and vegetables were purchased from six different retail outlets which were Community Market of Ahmadu Bello University (CM), Suleiman Hall (S.H), ICSA Ramat market (I.M), Social Centre (S.C), Danfodio Hall (D.H) and Samaru Market (S.M). All these sites are popular places where fruits and vegetables are bought by many people in Samaru. Altogether, a total of 108 samples were collected with the number of samples of each fruit and vegetable collected being 18 at each of the six retail outlets. Samples of each type of the vegetables and fruits bought from the different outlets were packed separately to avoid cross contamination. The samples were stored at 4ºC in sterilized polythene bags and were processed within 2 hours of collection.

Microbiological Procedures

Enrichment of Samples

Each sample of the vegetables and fruits was chopped into smaller pieces by using a sterilized knife and 10g was weighed aseptically. Each sample was transferred into stomacher bag containing 90ml of Peptone Water (PW) and mixed for one to two minutes. Thereafter, the PW primary mixtures (10^{-1}) were incubated at 37^0C for 18 to 24 hours.

Total Aerobic Plate Count

Enumeration of total viable bacteria was carried out using the total plate count (TPC) technique. A series up to 10^{-9} dilution was prepared by transferring 0.1ml of primary dilution into test tubes containing 9.9mls physiological saline. For the determination of TPC, 0.1ml of 10^{-9} dilution of the homogenate was inoculated into nutrient agar plate and spread using sterile glass spreaders. The petri dish was then kept in an incubator at 37^0C for 24 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The number of colonies in the dilution was multiplied by the dilution factor

to obtain the TPC. The TPC was expressed as the number of organisms of colony forming units per ml (CFU/ml) of samples as calculated using the formula below:
Number of bacteria per mL of serially diluted bacteria:

Number of CFU counted = Number of CFU/mLVolume plated (0.1mL) x dilution (10^{-10}) used

Total Coliform Count

Enumeration of total coliform bacteria was carried out using MacConkey agar by transferring 0.1ml of 10^{-7} dilution into MacConkey agar plate and spread using sterile glass spreaders. The petri dish was incubated at 37ºC for 24 hours. Thereafter, typical pinkish colonies were counted indicative of lactose fermenting bacteria while non-lactose fermenting bacteria appeared colourless. The counts were presented as colony forming units per ml (CFU/ml).

Isolation of *Escherichia coli*

The initial homogenate containing the buffered peptone water mixture was used. The medium for isolation of *E. coli* was Eosin-Methylene Blue (EMB) agar. A volume of 1ml of the pre enriched sample homogenate was transferred into dilution bottles containing 9ml of Tryptose soya broth and incubated at 37^0C for 24 hours. Then the sample was streaked onto EMB agar plate and incubated at 37^0C for 24 hours. Colonies that appeared greenish metallic sheen were identified as *E. coli*. These were inoculated onto nutrient agar slants and incubated for 24 hours at 37^0C and preserved in the refrigerator at 4^0C. Prior to biochemical test, the preserved isolates were sub-cultured onto EMB agar to obtain a pure culture at 37^0C for 24 hours. The sub-cultured colonies were then used for biochemical tests.

Biochemical Characterization

The characteristic colonies were identified on the basis of sugar utilization, IMVIC patterns and production of gas. The suspected *E. coli* colonies were tested for the ability to ferment lactose and sucrose in triple sugar iron (TSI) agar, slants for SIM medium (Sulphide, Indole and Motility), Methyl red and Voges proskaeur reaction (OXOID U.K), citrate utilisation (Oxoid, UK) and Urease Production (DIFCO laboratories, 1984) were utilized.

RESULTS

Total Aerobic Plate Count

Enumeration of bacterial count of the fruits and vegetables sold within Samaru, is shown in table 1. The mean total aerobic plate count (TAPC) ranged between 1.05 $\times10^{11}$ to 1. 64 $\times10^{11}$. The highest count (1.64 $\times10^{11}$) was in pineapple while the lowest (1.05 $\times10^{11}$) was in water melon. There was no much difference between the TAPC of lettuce (1.44 $\times10^{11}$) and cabbage (1.42 $\times10^{11}$) as well as between carrot (1.37 $\times10^{11}$) and cucumber (1.39 $\times10^{11}$). At the retail outlets, Samaru market had the highest bacterial count (1.56 $\times10^{11}$) followed by Community market (1.54$\times10^{11}$), Danfodio market (1.43 $\times10^{11}$), Social center (1.40$\times10^{11}$), ICSA market (1.38$\times10^{11}$) and Suleiman market had the least bacterial count (1.30 $\times10^{11}$) (table 2).

Total Coliform Count

The total coliform count is as shown in table 3. The mean coliform count ranged from 1.28$\times10^9$ to 3.55 $\times10^9$ CFU/ml. Carrot (1.28$\times10^9$) had the lowest count, followed by pineapple (2.02 $\times10^9$), cabbage (2.72 $\times10^9$), water melon (3.05 $\times10^9$), lettuce (3.17 $\times10^9$) and the highest was cucumber (3.55 $\times10^9$).

Isolation of *Escherichia coli*

Out of 108 samples obtained, 50 (46.3%) of the samples were suspected to be *E. coli*. On confirmation, only 23 (46%) out of the 50 samples suspected to be *E. coli* yielded reactions typical of *E. coli*. The overall prevalence of *E. coli* from the 108 fruits and vegetables in the study was 21.3%. The highest isolation of 5 (27.8%) was in cucumber and lettuce each, followed by 4 (22.2%) in cabbage, carrot and pineapple while the least was water melon 1 (5.6%) (table 4)

TABLE I: Enumeration of bacterial count of fruits and vegetables sold at selected retail outlets in Samaru, Kaduna State, Nigeria.

Type of Samples	Number of examined	Mean TAPC (CFU/ml)
Pineapple	18	1.64×10^{11}
Water Melon	18	1.05×10^{11}
Cucumber	18	1.39×10^{11}
Carrot	18	1.37×10^{11}
Cabbage	18	1.42×10^{11}
Lettuce	18	1.44×10^{11}

Key : TAPC Total Aerobic Plate Count

TABLE II: Enumeration of bacterial count from fruits and vegetables sold at selected retail outlets in Samaru, Kaduna State, Nigeria.

Location	Number of examined	Mean TAPC (CFU/ml)
Community market	18	1.54×10^{11}
Suleiman market	18	1.30×10^{11}
Samaru market	18	1.56×10^{11}
Icsa/Ramat market	18	1.38×10^{11}
Danfodio market	18	1.43×10^{11}
Social center	18	1.40×10^{11}

Key : TAPC Total Aerobic Plate Count

TABLE III: Total coliform count of fruits and vegetables sold at selected retail outlets in Samaru, Kaduna State, Nigeria.

Type of Samples	Number examined	Mean TCC (CFU/ml)
Pineapple	18	2.02×10^9
Water Melon	18	3.05×10^9
Cucumber	18	3.55×10^9
Carrot	18	1.28×10^9
Cabbage	18	2.72×10^9
Lettuce	18	3.17×10^9

Key: TCC = Total Coliform Count

TABLE IV: Prevalence of *Escherichia coli* in fruits and vegetables sold at selected retail outlets in Samaru, Kaduna State, Nigeria.

Samples	Number of Samples examined	Number of isolates	Number confirmed as *E .coli (%)*
Pineapple	18	9	4 (22.2)
Water Melon	18	10	1 (5.6)
Cucumber	18	8	5 (27.8)
Carrot	18	6	4 (22.2)
Cabbage	18	8	4 (22.2)
Lettuce	18	9	5 (27.8)
Total	108	50 (46.3%)	23 (21.3%)

Isolation of other Species of Coliforms

Table V shows the prevalence of other species of coliforms isolated from fruits and vegetables in this study. Six different species of coliforms were identified with *E. coli* having the highest rate (21.3%), followed by *Klebsiella* (8.3%), *Enterobacter* (7.4%), *Citrobacter* (5.5%), *Proteus* (2.7%) and *Serratia* (0.93%).

TABLE V: The prevalence of coliforms from the One hundred and eight vegetables and fruits samples sold at selected retail outlets in Samaru, Kaduna State, Nigeria.

Isolates	Number of isolates	Prevalence (%)
Escherichia coli.	23	21.3
Klebsiella spp	9	8.3
Enterobacter spp.	8	7.4
Citrobacter spp.	6	5.5
Proteus spp.	3	2.7
Serratia spp.	1	0.93
Total	50	46.13%

DISCUSSION

The result of this study indicates that all the samples were contaminated with bacteria as shown by the high microbial load which is higher than the standard bacterial count recommended by World Health Organization (WHO, 2006). The high total bacterial count may be attributed to the unhygienic practices right from the farm to the market and exposure to potential microbial contamination at every step, including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers (Heaton and Jones, 2008, Gultie and Sahile,2013, Ntuli *et al*., 2017). The high contamination might also indicate that the water used during planting or harvesting could be heavily contaminated with faecal matter from sewerage effluent. It has also been shown that high number of the family of Enterobacteriaceae on vegetables clearly proves that poor hygiene could be a source of foodborne pathogens (Weldezgina and Muleta, 2016). Routes of contamination are varied and include application of organic wastes to agricultural land as fertilizers, contamination of waters used for irrigation with faecal materials, direct contamination by livestock, wild animals, birds, and post-harvest issues such as workers hygiene, transport containers, harvesting equipment (Heaton and Jones, 2008). The results of this study is similar with the works of Uze *et al*., 2009, and Bukar *et al*., 2010, which both of them reported higher bacterial count found in commonly consumed fresh fruits and vegetables. Also, Ogbonna *et al*., 2010, reported the contamination of cabbage with *E. coli* and *Pseudomonas* species. The presence of these pathogens in ready to eat fruits and vegetables may cause gastroenteritis due to production of enterotoxins by the bacteria and sometimes may even cause the spoilage of the fruits and vegetables when their population is high (Adjrah *et al*., 2013, Ntuli *et al*., 2017). The high bacteria count seen in pineapple may be attributed to its fleshy nature that grows close to the ground.. Vegetables that grow closer to the ground are known to be more easily contaminated from soil microorganisms as compared to those that are high up the tree, far away from soil microbial contaminants (Bello *et al*., 2014). The high rate seen in fruits and vegetables collected from Samaru market may be attributed to the poor hygienic practices by the handlers and also the dirty water used in washing these products before they get to the final consumers (Ntuli *et al*., 2017).

Contamination of the vegetables and fruits by coliforms may be attributed to the wide distribution of coliforms in nature (Szabo & Coventry, 2001). Coliforms and faecal coliforms are usually indicators whose presence will normally indicate the presence of pathogenic organism, it gives a general indication of the sanitary condition of the fruits and vegetables. The 46.3% prevalence of coliforms is high as it exceeds the recommended limit (Gilbert, *et al*., 2000). The high count of coliforms in cucumber may be due to its fleshy nature which may make it more prone to bacterial penetration. On the other hand, the high coliform count in the examined vegetables and fruits may be attributed to the use of animal manures (Johannessen *et al*., 2005).

Escherichia coli was also isolated in all the samples with lettuce and cucumber having the highest (27.8%) and watermelon having the least (5.6%). This organism despite the fact that it is normal microflora of the intestinal tract of humans and other warm blooded animals, some strains are known to cause diarrheal illness (Cheesbrough, 1991). The presence of *E. coli* in these samples indicates the possibility of faecal contamination from manures and inferior post-harvest washing by processors to remove soil and debris (Jawetz, 2007). It may also be due to cross contamination by the handlers through poor hand washing, or contamination of utensils and preparation surfaces (Beuchat, 2002).

Water is mainly used for irrigation of plants (vegetables), which may be a source of contamination with the transfer of foodborne pathogenic microorganism from irrigation to vegetables. In general, the presence of *E.coli* in ready-to-eat foods is undesirable because it indicates poor hygienic conditions. The presence of non-faecal coliforms bacteria such as *Klebsiella, Enterobacter* , *Citrobacter, Proteus* and *Serratia* is not considered a public health threat but may result in spoilage of vegetables and fruits. Their presence on vegetables have long been recognized as common organisms. Therefore, level of faecal organisms, such as *E. coli*, is a better indicator of quality of fresh produce, and this could explain why this organism has been included as a hygienic criterion in the new EU regulation (Jeddi *et al*., 2014).

Even though none of the produces examined showed visible signs of damage, which indicates that out ward appearance may not be a good criterion for judging the microbial quality of fruits. So, all ready-to eat farm produces should be adequately washed before consumption to reduce microbial load (Gultie and Sahile,2013).

CONCLUSION

The results of this study showed that vegetables and fruits produced and consumed within Samaru were contaminated with bacteria and this may pose a great health hazard as these produce are sometimes consumed raw (vegetables) or without washing (fruits).

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