



Isolation of *Escheria Coli* from Fruits and Vegetables in Kaduna Metropolis

Bako Benjamin¹, Auwalu Uba², Muhammad Yusha'u³, D.B Maikaje⁴, Ninyio Nathaniel Nyakaat⁵, Akumba Martha Daniel⁶
Department of Microbiology^{1,2,3,4,5}, Department of Christian Religious Studies⁶
Kaduna State University, Kaduna, Nigeria¹
Abubakar Tafawa Balewa University, Bauchi, Nigeria²
Bayero University, Kano, Nigeria³
Kaduna State University, Kaduna, Nigeria^{4,5,6}

Abstract:

Three hundred (300) samples of fruits and vegetables were obtained from five (5) different Markets in Kaduna State Metropolis, Nigeria and screened for *Escherichia coli*. It was analyzed using the Spread Plate Method. The number of positive samples ranged between 6 -10 and the total number of positive samples is 38. The highest number of *Escherichia coli* was as a result of poor hygiene practice and handling. The microorganisms recovered was *Escherichia coli*. The percentage of contamination in each location is as follows: Tirkania 16.7%, Sabon-Tasha 15%, Central 10%, Kawo 11.7%, and Gonin-Gora 10%. The presence of this organism may pose a serious threats to human health *E. coli* has been reported to be responsible for the outbreaks linked to consumption of fresh but raw vegetables and fruits (such as lettuce, spinach, carrots, mango and cucumber etc.). These fruits and vegetables get contaminated with pathogenic *E. coli* while growing in the field or on application of fertilizer (cow dung) or during harvesting, transport, processing, storage and distribution. Therefore, measures should be taken by regulatory body in ensuring proper hygienic processing and handling techniques of fruits and vegetables being sold to general public.

Keywords: *Escherichia coli*, fruits, vegetables, outbreak and contamination.

1. INTRODUCTION

The gm-negative bacillus occurring singly or in pairs. *Escherichia coli* is facultatively anaerobic with both a fermentative and respiratory type of metabolism. It is either nonmotile or motile by peritrichous flagella. *E. coli* is a major facultative inhabitant of the large intestine and ubiquitous in human environment. It is one of the most frequent cases of the many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI) and traveler's diarrhoea and other clinical infections such as neonatal meningitis. Since 1885, *E. coli* has been recognized as both a harmless commensal and a versatile pathogen [2] Childhood diarrhoea is caused by ETEC as well as other bacterial and viral agents [29]. Food-borne diseases have been a real problem in both developed and developing countries in recent years and in addition to human pain, have caused major economic losses. It has been proved that in industrialized countries almost one third of the population is affected by food borne diseases each year, and the problem in developing countries is probably more [12]. In developed countries the data indicates 6 to 81 million cases of illness [1]. According to Fan *et al.*, (2009) "the total number of cases of foodborne illnesses in the United States has been estimated to be approximately 76 million illnesses per year, resulting in 325,000 hospitalizations and 5,000 deaths. In fact among all the crops, leafy vegetables present the highest concern regarding microbiological hazards at the moment. They have been connected with numerous epidemics with a high number of illnesses in at least three regions of the world and are grown and processed in diverse ways, like pre-cut and bagged products [11]. Only *E. coli* O157:H7 has caused illnesses in

more than 30 countries in different continents [11]. Nowadays minimally processed and ready to eat fruits and vegetables are considered as important part of a healthy diet. In fact there is an international movement to increase their consumption and, interestingly, the consumption of vegetables by consumers has increased by 8% from 1990 to 2005 [10]. The number of outbreaks of food borne diseases related to the consumption of fresh fruits and vegetables has augmented since the early 1990s. For example, in the USA between 1998 and 2002, 2.9% (192/6647) of total foodborne outbreaks were related to vegetables. Bacteria such as *E. coli* O157:H7 is previously related to illness from food of animal origin, has caused the highest proportion of epidemics associated with fresh produce that has an identified etiologic agent [3]. Numerous outbreak of gastrointestinal disease have been linked to consumption of fresh fruits and vegetables and the source of contamination can occur at various stages of production through actual sale of the final product [25] "Leafy green consumption between 1996 and 2005 increased by 9% compared to the previous decade, but outbreaks associated with leafy greens increased by 38.6%, with a majority of them caused by *E. coli* O157:H7 [10]. A reason for this is the belief that the daily consumption of fruits and vegetables will prevent certain diseases such as cardiovascular diseases and some cancers. Since 1885 *Escherichia coli* has been recognized as both harmless commensal and a versatile pathogen [2] often associated with contaminated water or food handlers. Fruits and vegetables normally carry both pathogenic and non-pathogenic strains of *Escherichia coli* [21]. Raw fruits and vegetables products have become some of the most scrutinized foods due to recent outbreaks associated with minimally processed produce. The most common route for fresh produce contamination is at the

preharvest stage, when microorganisms from animal feces, contaminated irrigation water, wild and domestic animals can be deposited in crops [9].

2. MATERIALS AND METHODS

2.1 Collection of samples

Sixty (60) samples of fruits and vegetables were aseptically collected from five (5) markets in Kaduna metropolis into sterile plastic bags and immediately transported to the laboratory and analyzed in the same day. A total of twelve (12) samples from each market of; Carrot, Cucumber, Lettuce, Spinach, Mango were collected in sterile polythene bags from Sabo market, Central market, Kawo market, Tirkania market, Gonin Gora market all within Kaduna metropolis.

2.1.1 Isolation of *Escherichia coli* from fruits and vegetables

Twenty five (25) grams of selected raw vegetables and fruits each were measured and finely chopped aseptically. The weighed samples were washed in 225ml of sterile distilled water, 1ml of the water was subjected to ten fold dilution

series. 1ml from 10^{-3} and 10^{-5} dilutions were spread plated in duplicate onto MacConkey Agar plates and the plates were incubated at 37°C for 24h. Identification of *Escherichia coli* Isolates. Cultural properties on MacConkey agar plates Morphological characteristics of colonies(size, shape, elevation, form, pigmentation) was carefully studied after which suspected *Escherichia coli* colonies were picked from the plate and sub-cultured onto fresh MacConkey plate and incubated at 37°C for 24h.

2.1.2 Screening of suspected *Escherichia coli* isolates

Suspected *Escherichia coli* isolates from fresh MacConkey plate were streaked onto Eosin methylene Blue (EMB) agar plate and incubated at 37°C for 24h for presumptive confirmation as *Escherichia coli*. The *Escherichia coli* colonies were black colonies with a metallic green sheen caused by large quantities of acid that is produced and that precipitates out the dyes onto the growth surface [5]. The bacterial isolates were then identified following standard microbiological procedures based on cultural, morphological and biochemical characteristics [4], [8] and [6].

3. RESULTS:

Table 1 shows the Location, type of market and type of samples.

L o c a t i o n	T y p e o f m a r k e t	T y p e o f s a m p l e s
S a b o	L o c a l	Mango, Lettus, Spinach, Carrot, Cucumber
Central	Mordern	Mango, Lettus, Spinach, Carrot, Cucumber
Tirkania	Local	Mango, Lettus, Spinach, Carrot, Cucumber
Gonin-gora	Local	Mango, Lettus, Spinach, Carrot, Cucumber
Kawo	Local	Mango, Lettus, Spinach, Carrot, Cucumber

The number of samples and the percentage of *Escherichia coli* contamination is shown in Table 2 below. The results of positive samples shows that 16.7%(10 samples) was the highest

obtained in Tirkania and 10%(6 samples) was the lowest which is obtained in Central Market and Gonin-Gora. The organism recovered is shown in Table 3.

Table 2 shows the Location, number of samples, number of positive samples and percentage of positive and negative samples.

L o c a t i o n	No of samples	No of positive samples	Percentage (%) of positive samples	No of Negative samples	Percentage (%) of Negative samples
S a b o	60	9	15	51	85
Central	60	6	10	54	90
Tirkania	60	10	16.7	50	83.3
Gonin-gora	60	6	10	24	90
Kawo	60	7	11.7	53	88.3

Table 3. Cultural and Biochemical Characteristic of *Escherichia coli* Isolated from fruits and vegetables.

C u l t u r a l C h a r a c t e r i s t i c s	T e s t	R e s u l t s
Black colonies with metallic green sheen	I n d o l e n t p r o d u c t i o n	+
	Methyl Red(MR)	+
	Voges proskauer(VP)	-
	Citrate utilization	-
	Glucose	+
	Sucrose	+
	Lactose	+
	Hydrogen sulphide	+
	Gas	+

4. DISCUSSION: The consumption of fresh produce has increased over last two decades due to an important source of

vitamins, nutrients and fiber [17]. In fact there is an international movement to increase their consumption and,

interestingly, the consumption of vegetables by consumers has increased by 8% from 1990 to 2005 [10]. The positive samples in Table 1 shows that 16.7% (10 samples) was the highest obtained in Tirkania and 10% (6 samples) was the lowest which is obtained in Central Market and Gonin-Gora. The percentage of positive samples in decreasing order are as follows: Tirkania 16.7% (10 samples), Sabon Tasha 15% (9 samples), Kawo 11.7%(7 samples), Gonin-Gora 10%(6 samples) and Central Market 10%(6 samples) which is due to increase in consumers awareness of health aspects of fresh vegetables and fruits as consumers are more concerned about correct eating habits and staying healthy [28]. The number of positive samples in Table 1 from Local markets are higher to that of the modern market this can be attributed to awareness of correct safety measures by farmers and consumers during pre-harvest (in the field), post-harvest and processing periods[26]. Similar to many of the developing and underdeveloped countries, the hygienic conditions in Kaduna metropolis are severely compromised and living with domestic animals within the same premises is a common practice among the Kaduna metropolis population. Therefore, *E. coli* and other resistant bacteria colonize these vegetables for a number of reasons; the direct use of antibiotics during cultivation, use of contaminated fertilizer or irrigation water or unknown human selection pressure [18]. Water is likely to be an important source of contamination in the field. Possible sources are runoff from nearby animal pastures and irrigation from a contaminated source [15; 24]. Insects are also a possible source of contamination. In laboratory conditions, contaminated flies have been shown to directly transfer bacteria to plant leaves or fruits [16; 22; 23]. Studies have implicated flies in contamination of leaves by *E. coli* O157:H7 [16]. Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of contamination [26]. Table 2. shows the number of *Escherichia coli* isolated in all the samples. Table 3. showed the percentages occurrence of *Escherichia coli* out of the three hundred (300) samples examined. The rate of the contamination by *Escherichia coli* is as follow: Sabo 9(15%), Central 6(10%), Tirkania 10(16.7%), Gonin-Gora 6(10%) and Kawo 7(11.7%). *Escherichia coli* is part of the normal flora of humans, its habitat includes water, sewage, food, animals and human intestine. Some strains can cause gastroenteritis, diarrhoea and urinary tract infection. Their presences are indicator of faecal contamination of fruits and vegetables. This organism is an opportunistic pathogen that can cause disease in immune compromised people, it cause diseases such as urinary tract infections (UTI), traveler's diarrhea, gastroenteritis cholecystitis, bacteremia, cholangitis and other clinical infections such as neonatal meningitis [2]. There are reports that provide the evidence that *E. coli* could be transmitted to vegetables from manure [27;19], contaminated irrigation water [13;20] and direct contamination from animal and human faeces [14] and through poor hygiene practice by the food handler [7].

5. CONCLUSION

It is now shown that *Escherichia coli* as an opportunistic pathogen is present in fruits and vegetables sold in markets within Kaduna Metropolis. Their presence is as a result of faecal contamination of the product due to watering these fruits and vegetables with contaminated water, lack proper hygiene during cultivation, transporting, processing and consumption.

Prevention strategies may include the use of hand washing sanitizer, controlled and supervised handling of raw and chopped vegetables and clear separation of food-related activities from the areas of housing animals. All these may help to reduce the risk of transmission of this pathogen though their presence does not cause disease in healthier humans except in immune suppressed and debilitated people.

6. REFERENCES

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