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Original Research Article

Enumeration, Isolation and Identification of Bacteria Isolated from Irrigated Vegetables in some Selected Area of Kano Metropolis, Kano Nigeria

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Abstract: Vegetables are dietary sources of nutrient, vitamins and fiber for human growth and development cultivated with irrigation water and sewage which can cause contamination. Therefore, the study was aimed to enumerate, isolate and identify bacteria isolated from irrigated vegetables in Kano metropolis. A total of twenty seven 27 vegetables samples were collected from three kinds of vegetables (Carrot, Lettuce and Cabbage) from three different locations (Kwakwaci, Shagari and Jaba quarters wastewater irrigation area). The mean bacterial counts for *Daucus carota* (Carrot) sample based on the three different location (Kwakwaci, Shagari and Jaba wastewater irrigation area) ranged from 1.40 x10^3 to 1.63 x10^3 cfu/ml, the mean bacterial counts for Cabbage sample range from 1.90 x10^3 to 4.43 x10^3 while the mean of bacterial counts for *Latuca sativa* (Lettuce) sample range from 1.33 x10^3 to 1.70 x10^3. In Gram-negative bacteria, the most common bacteria isolated and identified were *Escherichia coli* (27.54%) followed by *Salmonella* (24.64%) and *Shigella* (17.39%). In the Gram-positive bacteria only *Staphylococcus aureus* (30.43%) was found.

Keywords: Bacteria, Irrigation, Kano, Lettuce, Vegetables.

INTRODUCTION

More than 10% of the world's population consumes foods produced by irrigation with wastewater (WHO, 2006). Wastewater reuse in irrigation is largely considered an inevitable option to compensate water shortages in developing countries. Hence, crop irrigation with wastewater is a widespread practice in these countries (Sou *et al.*, 2011). One of the most economically feasible agricultural uses of reclaimed water is the irrigation of vegetables which typically has high returns per volume of water invested in it (Toze *et al.*, 2006). Although, the environmental and public health risks posed by wastewater irrigation are alarming, especially when untreated and/or partially treated wastewater is used for such purposes (Ackerson *et al.*, 2012).

The point sources of contamination to water bodies include industrial effluents, municipal/domestic wastewater, abattoir waste, while, nonpoint sources include wild animal defecation, storm water drainage and urban runoff (Odjadjare *et al.*, 2010). The pathogens can be transmitted to the public through consumption of irrigated produce, especially crops eaten raw (Blumenthal *et al.*, 2000). Several studies throughout the world have demonstrated a very close relation between the consumption of fruits and vegetables irrigated with raw wastewater and many food borne diseases like gastroenteritis, cholera, chemical toxicity etc. (Sou *et al.*, 2011). Fresh and minimally processed vegetables and fruits provide the most important human diet that contains carbohydrates, proteins, vitamins, minerals, fiber and other benefits (Ijabadeniyi, *et al.,* 2010).

Their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes, and cancer has resulted in a further increase in desirability and consumption. For instance, Food and Drug Administration (FDA) and World Health Organization (WHO) have recommended 5–9 servings of fruits and vegetables to be taken daily due to the fact that correct fresh produce intake alone could save 2.7 million lives a

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year. In contrast to their health benefits, the consumption of fresh fruits and vegetables has also been associated with increasing of risk for consumers and outbreaks of food infections associated with consumption (Desta and Diriba, 2016).

Garg *et al.,* (2010); Zhao *et al.,* (2014) 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. Furthermore, surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including *Salmonella* spp., *Shigella* spp., Shiga toxigenic *E. coli* (STEC), *Listeria monocytogenes, Campylobacter* spp. *Escherichia coli* O157:H7, *Staphylococcus aureus, Bacillus cereus, Vibrio* spp., *Clostridium perfringens, Enterococcus* spp. *and Streptococcus* spp. are among the most common foodborne pathogens, affecting millions of people annually which results in symptoms range from mild to severe, and include fever, headache, nausea, vomiting, abdominal pain and diarrhea, and sometimes organ failure and death (McCabe *et al.,* 2019). Outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased, despite their nutritional and health benefits (Baishakhi *et al.,* 2020). Therefore, the study was aimed to enumerate, isolate and identify bacteria isolated from irrigated vegetables in Kano metropolis.

MATERIAL AND METHODS

Study Area

Samples were collected from three different Local Governments of Kano metropolis which are; Fagge (Kwakwaci wastewater irrigation area), Kumbotso (Shagari quarters wastewater irrigation area) and Ungoggo (Jaba wastewater irrigation area).

Sample Collection

Twenty Seven (27) samples were collected randomly directly from each sampling site a in polythene bags. The samples were immediately taken to the laboratory in an iced box for further analysis (Baker and Silverton, 2007).

Aerobic Mesophilic Bacterial Counts

This was carried out by pour plating technique and serial dilution (Egboh and Emeshili, 2007). Ten grams (10g) of the sample was transferred into a conical flask containing 90ml of buffered peptone water (BPW), using sterile pipette syringe one millimeter was transferred into first test tube as 10^{-1} containing 9ml of BPW (using a separate syringe), this was repeated to obtained 10^{-2} to 10^{-5}. Using another fresh syringe, 1ml of sample from each dilution was transferred into two sterile petri dishes and labeled accordingly. This was followed by pouring of cooled molten nutrient agar in each petri dish, swirled clockwise and anti-clockwise and allowed to solidify. Finally the plates were incubated at 37^0C for 24hours. After 24 hours of incubated plates was between 30-300 colonies were selected and counted to get number cfu/g.

Isolation of Spoilage Microorganisms

Organisms were determined by inoculation on; MacConkey agar (MAC), Eosine Methylene Blue agar (EMB), *Salmonella-Shigella* agar (SSA), Mannitol Salt agar (MSA) and Blood agar respectively. This was further confirmed with biochemical test as described by (Cheesbrough, 2010).

Identification of Bacteria Isolates

Gram staining of the Bacterial Isolates

Thin smear was made on a clean-grease-free glass slide which was fixed by passing over a bunsen flame. A crystal violate solution was applied to cover the smear for 30 seconds and was washed with distilled water. Secondly, Lugol's iodine was applied to the surface for 30 seconds. Acetone was used as a decolorizer and applied briefly. Safranin solution was applied on the surface for 30 seconds this was also washed and allowed to air dry at room temperature. The stain was observed under microscope after oil immersion has been applied onto the slides. Consequently, red stain indicates Gram-negative and purple stain indicates Gram-positive bacteria (Cheesbrough, 2010).

Biochemical Tests for Characterization of Bacteria

The bacterial isolates were characterized on the basis of their colonial morphology, cellular morphology through Gram staining and biochemical characteristics such as catalase, coagulase, citrate utilization, methyl red, VP, indole, and triple sugar iron tests as demonstrated by (Cheesbrough, 2010).

RESULTS

Aerobic Mesophilic Bacterial Counts of Carrots

results of aerobic mesophilic bacterial counts of the carrot samples examined showed that sample S- Car^3 had the highest average count of (1.9 x10^3CFU/g) while sample K- Car^3 had the least average count of (1.0 x10^3CFU/g) (Table 1).

Table 1: Aerobic Mesophilic bacterial counts of the Carrot samples

Sample Code	Bacterial Counts (cfu/g)	FAO Standard
K- Car^1	1.4 x10^3	1.0 x 10^5
K- Car^2	1.8 x10^3	
K- Car^3	1.0 x10^3	

Sample Code	Bacterial Counts (cfu/g)	FAO Standard
S- Car^1	1.4 x10^3	
S- Car^2	1.6 x10^3	
S- Car^3	1.9 x10^3	
J- Car^1	1.1 x10^3	
J- Car^2	1.8 x10^3	
J- Car^3	1.4 x10^3	

Key: S/N =serial number, cfu/g =colony forming unit per gram, MPN/g =most probable number per gram, K-Car$^{1-3}$ = kwakwaci carrot, S-Car$^{1-3}$ = Shagari Carrot, J-Car$^{1-3}$ = Jaba Carrot.

Bacterial Count of Carrots Based on Locations

The mean of bacterial count based on three different locations from the samples range from 1.40 x10^3 to 1.63 x10^3 Cfu/ml. The highest bacterial count was observed in sample S-Car$^{1-3}$ (1.63 x10^3) while the least Count was observed in sample K-Car$^{1-3}$ (1.40 x10^3) (Table 2).

Table 2: Mean Aerobic Mesophilic bacterial counts of Carrot samples based on the location

Sample Code	Bacterial Counts (cfu/g)	FAO Standard
K-Car$^{1-3}$	1.40 x10^3 ±40.00^a	1.0 x 10^5
S-Car$^{1-3}$	1.63 x10^3 ±25.67^b	
J-Car$^{1-3}$	1.43 x10^3 ±35.18^a	

Key: S/N =serial number, cfu/g =colony forming unit per gram, K-Car$^{1-3}$ = kwakwaci carrot, S-Car$^{1-3}$ = Shagari Carrot, J-Car$^{1-3}$ = Jaba Carrot. During the study, the mean bacterial counts of the three different locations were compared to establish any significant difference. The mean difference is significant at 0.05

Aerobic Mesophilic Bacterial Counts of Cabbage

The results of aerobic mesophilic bacterial counts of the cabbage samples examined showed that sample S-Cab^1 had the highest average count of (9.0 x10^{3}CFU/g) while sample K-Cab^3 had the least average count of (1.2 x10^{3}CFU/g) (Table 3).

Table 3: Aerobic Mesophilic bacterial counts of Cabbage

Sample Code	Bacterial Counts (cfu/g)	FAO Standard
K-Cab^1	2.8 x10^3	1.0 x 10^5
K-Cab^2	1.7 x10^3	
K-Cab^3	1.2 x10^3	
S-Cab^1	9.0 x10^3	
S-Cab^2	1.9 x10^3	
S-Cab^3	2.4 x10^3	
J-Cab^1	2.3 x10^3	
J-Cab^2	2.5 x10^3	
J-Cab^3	2.6 x10^3	

Key: S/N =serial number, cfu/g =colony forming unit per gram, MPN/g =most probable number per gram, K-Cab$^{1-3}$ = Kwakwaci Cabbage, S-Cab$^{1-3}$ = Shagari Cabbage and J-Cab$^{1-3}$ = Jaba Cabbage.

Bacterial count of Cabbage Based on Locations

The mean of bacterial count based on three different locations from the samples. The highest bacterial count was observed in sample S-Cab$^{1-3}$ (4.43 x10^3) while the least Count was observed in sample K-Cab$^{1-3}$ (1.90 x10^3) (Table 4).

Table 4: Mean Aerobic Mesophilic bacterial counts of Cabbage samples based on the location

Sample Code	Bacterial Counts (cfu/g)	FAO standard
K-Cab$^{1-3}$	1.90 x10^3 ±82.51^c	1.0 x 10^5
S-Cab$^{1-3}$	4.43 x10^3 ±37.74^a	
J-Cab$^{1-3}$	2.47 x10^3 ±15.75^b	

Key: S/N =serial number, cfu/g =colony forming unit per gram, K-Cab$^{1-3}$ = Kwakwaci Cabbage, S-Cab$^{1-3}$ = Shagari Cabbage and J-Cab$^{1-3}$ = Jaba Cabbage. During the study, the mean bacterial counts of the three different locations were compared to establish any significant difference. The mean difference is significant at 0.05 levels

Aerobic Mesophilic Bacterial Counts of Lettuce

The results of aerobic mesophilic bacterial counts of the lettuce samples examined showed that sample K- Let^{2} had the highest average count of (2.2 x10^{3}CFU/g) while sample K- Let^{1} had the least average count of (1.0 x10^{3}CFU/g) (Table 5).

Table 5: Aerobic Mesophilic bacterial counts of Lettuce samples

Sample Code	Bacterial Counts (cfu/g)	FAO standard
K- Let^{1}	1.0 x10^{3}	1.0 x 10^{5}
K- Let^{2}	2.2 x10^{3}	
K- Let^{3}	1.9 x10^{3}	
S- Let^{1}	1.6 x10^{3}	
S- Let^{2}	1.2 x10^{3}	
S- Let^{3}	1.9 x10^{3}	
J- Let^{1}	1.2 x10^{3}	
J- Let^{2}	1.1 x10^{3}	
J- Let^{3}	1.7 x10^{3}	

Key: S/N =serial number, cfu/g =colony forming unit per gram, MPN/g =most probable number per gram, K-Let$^{1-3}$ Kwakwaci Lettuce, S- Let$^{1-3}$ = Shagari Lettuce and J- Let$^{1-3}$ = Jaba Lettuce

Bacterial count of Lettuce Based on Locations

The mean of bacterial count based on three different locations from the samples. The highest bacterial count was observed in sample K- Let$^{1-3}$ (1.70 x10^{3}) while the least Count was observed in sample J- Let$^{1-3}$ (1.33 x10^{3}) (Table 6).

Table 6: Mean Aerobic Mesophilic bacterial counts of Lettuce samples based on the location

Sample Code	Bacterial Counts (cfu/g)	FAO Standard
K- Let$^{1-3}$	1.70 x10^{3} ±624.^{b}	1.0 x 10^{5}
S- Let$^{1-3}$	1.56 x10^{3} ±351.2^{a}	
J- Let$^{1-3}$	1.33 x10^{3} ±321.5^{a}	

Key: S/N =serial number, cfu/g =colony forming unit per gram, K-Let$^{1-3}$ Kwakwaci Lettuce, S- Let$^{1-3}$ = Shagari Lettuce and J- Let$^{1-3}$ = Jaba Lettuce.

Morphological and Biochemical Characteristics of Bacteria isolated from Vegetables

Table 7 shows the morphological and biochemical characteristics for the identification of the bacterial species obtained from the samples examined. From the results, 4 different isolates were identified namely; *Salmonella, Staphylococcus aureus, Escherichia coli* and *Shigella.*

Table 7: Morphological and Biochemical characteristics of Bacteria isolated from Vegetables

Morphological characteristics	Gram Reaction	1	2	3	4	5	6	Isolates
Large mucoid growth on Mac. Agar	Gram negative short rod	+	-	+	-	-	+	*Salmonella*
Smooth circular, yellow colonies on MSA	Gram positive cocci in cluster	+	+	-	+	+	+	*S. aureus*
Green metallic shine on EMB	Gram negative rods	+	-	+	-	-	+	*E. coli*
Clear colorless transparent colonies on SSA	Gram negative pink short rods in singles	+	+	-	+	+	+	*Shigella*

Key: Mac. Agar = MacConkey agar, MSA= Mannitol salt agar, EMB= Eosine methylene blue agar, (1) = Catalase (2) = Coagulase (3) = Indole (4) = Voges proskauer (5) = citrate and (6) = methyl red.

Occurrence of Bacteria Isolates from the Vegetables Samples

The isolated bacteria were; *Shigella, Salmonella, Escherichia coli* and *Staphylococcus aureus,* among the bacterial isolated, *Staphylococcus aureus* had the highest percentage of occurrence of 30.43% while *Shigella* had the least percentage of occurrences of 17.39% (Table 8).

Table 8: Frequency of occurrence of bacteria Isolates from the Vegetables samples

S/N	S/C	*Shigella*	*Salmonella*	*E. coli*	*S. aureus*
1.	K-Car^{1}	+	+	+	+
2.	K-Car^{2}	-	+	+	+
3.	K-Car^{3}	-	-	+	+
4.	S-Car^{1}	+	-	+	-

S/N	S/C	*Shigella*	*Salmonella*	*E. coli*	*S. aureus*
5.	S-Car^{2}	-	-	+	+
6.	S-Car^{3}	+	+	+	+
7.	J-Car^{1}	-	-	-	+
8.	J-Car^{2}	-	+	-	+
9.	J-Car^{3}	+	-	+	+
10.	K-Cab^{1}	-	+	+	+
11.	K-Cab^{2}	-	-	+	+
12.	K-Cab^{3}	-	-	+	+
13.	S-Cab^{1}	+	+	+	+
14.	S-Cab^{2}	-	+	-	+
15.	S-Cab^{3}	-	-	-	+
16.	J-Cab^{1}	-	+	+	-
17.	J-Cab^{2}	+	+	-	+
18.	J-Cab^{3}	+	+	+	+
19.	K-Let^{1}	-	+	+	-
20.	K- Let^{2}	+	+	-	+
21.	K- Let^{3}	+	+	-	+
22.	S- Let^{1}	-	+	+	+
23.	S- Let^{2}	+	-	+	-
24.	S- Let^{3}	-	+	-	+
25.	J- Let^{1}	+	+	+	+
26.	J- Let^{2}	+	-	+	-
27.	J- Let^{3}	-	+	+	-
28.	**Total**	**12 (17.39%)**	**17 (24.64%)**	**19 (27.54%)**	**21 (30.43%)**

Key: S/N=serial number, S/C=sample code, *E. Coli = Escherichia coli, S. aureus = Staphylococcus aureus,* + = Positive, - = Negative, K-Car$^{1-3}$ = kwakwaci carrot, S-Car$^{1-3}$ = Shagari Carrot, J-Car$^{1-3}$ = Jaba Carrot, K-Cab$^{1-3}$ = Kwakwaci Cabbage, S-Cab$^{1-3}$ = Shagari Cabbage, J-Cab$^{1-3}$ = Jaba Cabbage, K-Let$^{1-3}$ Kwakwaci Lettuce, S- Let $^{1-3}$ = Shagari Lettuce and J- Let $^{1-3}$ = Jaba Lettuce.

DISCUSSION

In this study, the maximum mean bacterial count observed for both the vegetables samples (Carrot, Cabbage and Lettuce) was 9.0 x10^{3}cfu/g there has been a large debate concerning the total viable bacterial count in foods and food products at the point of consumption. FAO, (2020) that is 10^{5}cfu/ml, hence during the study, the vegetables samples (Carrot, Cabbage and Lettuce) supplied by the three different locations was fit for Human consumption.

The bacterial count in this study is relatively higher than previous reports from Morocco, Ibenyassine *et al.*, (2007), but other studies reported a lower count that ranged from 2 to 6 log CFU g^{-1} (Kwashie, 2009). Generally, there is no specification set for the permissible level of microbes for raw food being served in Ethiopia. However, Hazard Analysis and Critical Control Points-Total Quality Management (HACCP-TQM) Technical Guidelines lay down the microbial quality for raw foods, where the food containing less than 4, 4–6.69, 6.69–7.69 and greater than 7.69 log CFU g^{-1} (aerobic plate count) is rated as good, average, poor, and spoiled food, respectively (Aycicek *et al.*, 2016).

The overall mean counts of total and fecal coliforms from vegetable samples in the present study are relatively lower than Nipa *et al.*, (2011), who reported >1100 MPN 100 mL^{-1} from salad vegetables. In addition, the current finding is lower than the results obtained by Ashenafi (1989), who reported total coliform 1.5 × 10^{3} MPN 10 g^{-1} and fecal coliform 3.7 × 10^{2} MPN 10 g^{-1} counts of raw consumed food like tomato in Ethiopia. A survey carried out on spring onions, lettuce, and cabbage cultivated with poor quality irrigation water in Ghana also showed heavy contamination with fecal coliform between 4.0 × 10^{3} and 9.3 × 10^{8} MPN g^{-1} (2006). The observed difference in counts can be attributed in part to the degree of original contamination, storage conditions, and the hygienic conditions of utensils and vegetables handlers.

According to the study, bacteria associated with vegetables samples include *Shigella, Salmonella, Escherichia coli* and *Staphylococcus aureus.* The prevalence of *S. aureus* in the current study in all vegetables was lower than what is mentioned in the study of Halablab *et al.*, (2011) who reported higher prevalence of *S. aureus* (51.5%) from Lebanon. In addition, Ijabadeniyi (2010) also reported 67.0% in broccoli and 33.0% in cauliflower in South Africa. The presence of low *S. aureus* (20.0%) in the irrigation water in this study was of lower results than those obtained by Ikpeme *et al*. (2011) (25–33%) from two rivers that are used for irrigation of vegetables in South Africa. *Shigella* was isolated from vegetable samples tested in the current study. This is in line with Guchi and Ashenafi (2010) reported *Shigella* isolates from 8 (12.5%) samples of lettuces and 16 (25.0%) samples of green peppers from super market of Addis Ababa, Ethiopia. In the present study, the prevalence of *Salmonella* spp. in all vegetable samples was higher than in other reports by Guchi and Ashenafi (2010) and Ijabadeniyi (2010) who reported 10% in lettuce and green peppers as well as 11% in broccoli and cauliflower, respectively.

CONCLUSION

The work confirmed that vegetables samples (Carrot, Cabbage and Lettuce) from Fagge (Kwakwaci wastewater irrigation area), Kumbotso (Shagari quarters wastewater irrigation area) and Ungoggo (Jaba wastewater irrigation area), Kano State Nigeria is safe and acceptable for consumption as the bacterial load was within the limit set by FAO, (2020) (10^5 cfu/ml). However, the bacterial isolated and identified in the study were; *Shigella, Salmonella*, *Escherichia coli* and *Staphylococcus aureus*.

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